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A Novel Sucrose Synthase Pathway for Sucrose Degradation in Cultured Sycamore Cells[1]

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STEVEN C. HUBER*[2] AND TAKASHI AKAZAWA
Research Institute for Biochemical Regulation, School of Agriculture, Nagoya University, Chikusa, Nagoya 464, Japan

ABSTRACT

Enzymes of sucrose degradation and glycolysis in cultured sycamore (*Acer pseudoplatanus* L.) cells were assayed and characterized in crude extracts and after partial purification, in an attempt to identify pathways for sucrose catabolism. Desalted cell extracts contained similar activities (20–40 nanomoles per milligram protein per minute) of sucrose synthase, neutral invertase, glucokinase, fructokinase, phosphofructokinase, and UDPglucose pyrophosphorylase (assayed with 2 micromolar pyrophosphate (PPi). PPi-linked phosphofructokinase activity was virtually dependent upon fructose 2,6-bisphosphate, and the maximum activity exceeded that of ATP-linked phosphofructokinase. Hexokinase activity, with glucose as substrate, was highly specific for ATP, whereas fructokinase activity was relatively nonspecific. At 1 millimolar nucleoside triphosphate, fructokinase activity decreased in the order: UTP > ATP > CTP > GTP. We propose two pathways for sucrose degradation. One involves invertase action, followed by classical glycolysis of hexose sugars, and the other is a novel pathway initiated by sucrose synthase. The K_m for sucrose of sucrose synthase was severalfold lower than that of neutral invertase (15 *versus* 65 millimolar), which may determine carbon partitioning between the two pathways. The sucrose synthase pathway proposed involves cycling of uridylates and PPi. UDPglucose pyrophosphorylase, which is shown to be an effective 'PPi-scavenger,' would consume PPi and form UTP. The UTP could be then utilized in the UTP-linked fructokinase reaction, thereby forming UDP for sucrose synthase. The source of PPi is postulated to arise from the back reaction of PPi-linked phosphofructokinase. Sycamore cells contained a substantial endogenous pool of PPi (about 3 nanomoles per gram fresh weight, roughly 1/10 the amount of ATP in these cells), and sufficient fructose 2,6-bisphosphate (0.09 nanomole per gram fresh weight) to activate the PPi-linked phosphofructokinase. Possible regulation and energetic differences between the sucrose synthase and invertase pathways are discussed.

Sucrose is the primary transport form of reduced carbon in many higher plants. The sucrose is translocated from its site of synthesis in mature leaves to various metabolic 'sink' tissues, where it is used to support growth and synthesis of reserve materials such as starch. Identifying the possible limitations to growth of different plant organs is of considerable interest and agronomic importance. A prerequisite to understanding 'sink strength' at the biochemical level is fundamental knowledge of the pathway(s) of sucrose metabolism. At present, the pathways involved in sucrose degradation and conversion to starch are not clearly defined.

Degradation of sucrose within plant cells is thought to involve sucrose synthase and/or invertase (neutral or alkaline forms) (17). The invertase reaction produces glucose and fructose, which must be phosphorylated prior to subsequent metabolism. In higher plants, phosphorylation of glucose involves hexokinase (nonspecific) or glucokinase (2, 20), whereas phosphorylation of fructose is apparently catalyzed only by a specific fructokinase (21).

In order for sucrose synthase to participate in sucrose degradation for glycolysis, a source of UDP must be present and a mechanism must exist to metabolize the UDPG[3] formed. Three possibilities exist for the subsequent metabolism of UDPG. They are: UDPG pyrophosphorylase, UDPG phosphorylase, and phosphodiesterase, which catalyze the following reactions:

a) UDPG + PPi⇌glucose 1-P + UTP

b) UDPG + Pi→glucose 1-P + UDP

c) UDPG + H_2O→glucose 1-P + UMP

Coupling of sucrose synthase and UDPG pyrophosphorylase (reaction a) has been postulated in the past (4–6, 15), but at that time, the existence of a PPi pool in plant tissues was unknown. It was generally accepted that inorganic pyrophosphatase kept the PPi pool very low. Recently, substantial PPi pools have been detected in pea (7, 19) and corn (19) seedlings. However, it has not been demonstrated whether the UDPG pyrophosphorylase reaction is sufficiently active with the measured concentrations of PPi to serve as an effective PPi-scavenger *in vivo*. UDPG phosphorylase (reaction b) has been reported in potato tubers (8), but the distribution of this novel enzyme has not been investigated. However, it is an intriguing possibility because Gibson and Shine (8) reported that the enzyme was regulated by F26BP. Phosphodiesterase (reaction c) is not commonly considered to be involved in carbohydrate metabolism, but could conceivably function in UDPG metabolism (16).

Cultured plant cells are a useful system for a variety of biochemical studies, and have been used to study carbohydrate metabolism (10, 11). The cells, which are heterotrophic and contain numerous starch-filled amyloplasts, are generally grown on a sucrose-containing medium. Thus suspension-cultured cells may provide a model system for studies of carbohydrate metab-

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[2] Permanent address: United States Department of Agriculture, Agricultural Research Service, and Departments of Botany and Crop Science, North Carolina State University, Raleigh, NC 27695.

[3] Abbreviations: ADPG, ADPglucose, UDPG UDPglucose, F26BP, fructose 2,6-bisphosphate; FPLC, fast protein liquid chromatography.

olism in "sink" tissues. MacDonald and ap Rees (10), using cultured soybean cells, studied the intracellular compartmentation of enzymes in an effort to determine the form of carbon transported into the amyloplast. They concluded that sucrose is converted to triose-P in the cytosol. Although the focus of their study was on amyloplast enzymes, they postulated that sucrose was degraded by invertase to free hexose sugars. Hexokinase activity was not measured, but was presumed to be present, thereby forming hexose-P. The cytosol was shown (10) to contain the glycolytic enzymes necessary to convert hexose-P to triose-P. The cultured soybean cells also contained substantial activities of sucrose synthase (10), but the possible function of this enzyme was not considered.

The overall goal of the present study was to determine whether sucrose synthase may participate in sucrose degradation in cultured cells of sycamore (*Acer pseudoplatanus* L.). The approach taken was to determine whether the cells contained enzymes capable of metabolizing the products of the reaction, *i.e.* fructose and UDPG. We propose a novel sucrose synthase pathway that involves cycling of PPi and uridylates, and converts sucrose to triose-P.

MATERIALS AND METHODS

Plant Material. Suspension-cultured cells of sycamore (*Acer pseudoplatanus* L.) were grown as described by Bligny (1). The cells used in this study were harvested during the exponential growth phase.

Preparation of Cell Extracts and Column Chromatography. Cells (about 10 g fresh weight) were disrupted by sonication (200 W, 60 cycles/min) for 1 min in 20 ml of buffer containing 50 mM Tris-HCl (pH 8.0), 1 mM $MgCl_2$, 1 mM EDTA, and 10 mM KCl. After centrifugation at 10,000*g* for 10 min, the supernatant was desalted by passage through a Sephadex G-25 column equilibrated with 50 mM Tris-HCl (pH 8.0) and 1 mM EDTA. Part of the desalted crude extract (containing 3–4 mg protein/ml) was saved for enzyme analyses, and the remainder (25 mg protein) was fractionated by ion exchange chromatography using a Pharmacia FPLC system with a Mono Q column. The matrix was equilibrated with 50 mM Tris-HCl (pH 8.0) and 1 mM EDTA. After the sample was applied, the column was washed with the same buffer until the A_{280} approached zero. Proteins were eluted with a linear KCl gradient, as specified in the text. The FPLC was conducted at 25°C; all other steps were at 0 to 4°C.

Enzyme Assays. All assays were conducted spectrophotometrically (25°C) at 340 nm. The basic reaction mixture (1 ml) for all assays contained 50 mM Hepes-KOH (pH 7.0), 2 mM $MgCl_2$, 1 mM EDTA, 15 mM KCl, and other additions as specified for each enzyme. All coupling enzymes were desalted before use. (a) Invertase, 0.4 mM NAD, 2 units/ml glucose 6-P dehydrogenase (Sigma G-5885), 4 units/ml phosphoglucoisomerase, 4 units/ml yeast hexokinase, and 1 mM Mg·ATP. Reactions were initiated by addition of 25 mM sucrose. The glucose 6-P dehydrogenase used in all enzyme assays was prepared from *Leuconostoc mesenteroides*. This enzyme is active with either NAD or NADP; however, using NAD eliminates the need to correct for activity of 6-P gluconate dehydrogenase that may be present in the enzyme sample. (b) Sucrose synthase, assayed in the breakdown direction as UDP (1 mM)-dependent formation of hexose sugars from sucrose. Reaction conditions as described for invertase. (c) UDPG pyrophosphorylase, 0.4 mM NAD, 2 units/ml glucose 6-P dehydrogenase, 2 units/ml phosphoglucomutase, and 1 mM UDPG. Reactions were initiated by the addition of PPi (concentration indicated in the text). (d) UDPG or ADPG phosphorylase, conditions as in (c) except that 10 mM phosphate replaced PPi, and activity was assayed in the presence and absence of F26BP. (e) Glucokinase and fructokinase, 0.4 mM NAD, 2 units/ml glucose 6-P dehydrogenase, 2 units/ml phosphoglucoisomerase, 1 mM Mg·ATP and 2 mM glucose or fructose. Reactions were initiated by addition of hexose. (f) PPi-phosphofructokinase, 1 mM fructose 6-P, 3 units/ml aldolase, 3 units/ml α-glycerophosphate dehydrogenase, 6 units/ml triose-P isomerase, 0.2 mM NADH, 0.5 mM Mg·PPi, and 20 μM F26BP. (g) Phosphofructokinase, conditions as in (f), except that 1 mM Mg·ATP replaced PPi and F26BP was omitted.

Extraction and Analysis of Metabolites. Cells were collected by filtration through Miracloth, washed twice with 0.4 M mannitol, and the fresh weight recorded prior to freezing approximately 5 g samples in liquid N_2. Extraction for analysis of PPi was essentially as described by Smyth and Black (19). Each 5 g cell sample was ground in a mortar with 10 ml of 0.45 N $HClO_4$. The homogenate as centrifuged at 2000*g* for 5 min. An aliquot (4.5 ml) of the supernatant was centrifuged again in a Tomy MC-15A microfuge for 30 s. The supernatant was neutralized with KOH, and the resultant precipitate removed by microfuge centrifugation. Analysis of the neutralized extracts for PPi (7) and ATP (19) was exactly as described in the references cited. Metabolite recovery was tested by adding either 50 nmol of PPi or 200 nmol of ATP to the buffer prior to tissue extraction.

Separate samples were extracted and analyzed for F26BP. The extraction buffer contained 0.1 M Tris-HCl (pH 8.0), 5 mM EDTA, 20 mM KF, and 10 mM sodium phosphate. Cells (5 g) were ground in a mortar with 10 ml of extraction buffer. The homogenate was then heated at 80°C for 5 min, followed by centrifugation in a Tomy microfuge for 30 s. The concentration of F26BP was measured by the stimulation of potato tuber PPi-phosphofructokinase (Sigma F2258), in a coupled system containing 1 mM fructose-6-P, 0.2 mM NADH, 2 units/ml aldolase, 3 units/ml α-glycerophosphate dehydrogenase, and 6 units/ml triose-P isomerase. Reaction mixtures (1 ml), containing up to 0.2 ml of cell extract, were preincubated for 2 min at 25°C prior to initiating the assay by addition of 0.5 mM Mg·PPi. Standard curves were run in the presence of the cell extract, and recovery was determined by adding 2 nmol of F26BP to the extraction buffer.

RESULTS

Enzyme Activities in Cell Extracts. The activities of selected enzymes of carbohydrate metabolism in desalted extracts of sycamore cells are presented in Table I. The extracts contained substantial and generally similar activities of sucrose synthase and invertase, both assayed at pH 7.0. Activity of sucrose phosphorylase was undetected, as would be expected (data not shown). Because free sugars are products of sucrose synthase (fructose) and invertase (glucose plus fructose), we assayed cell extracts for hexose kinase activities. The extracts catalyzed the

Table I. *Activities of Enzymes of Carbohydrate Metabolism in Desalted Sycamore Cell Extracts*

Values are means of two experiments.

Enzyme	Additions	Activity
		nmol/mg protein·min
Sucrose synthase		19
Neutral invertase		14
Hexokinase (glucose)		17
Fructokinase		15
UDPG pyrophosphorylase	+ 2 μM PPi	39
	+ 1 mM PPi	196
UDPG phosphorylase	± 20 μM F26BP	0.5
ADPG phosphorylase	± 20 μM F26BP	0.5
PPi-phosphofructokinase	− F26BP	2
	+ 20 μM F26BP	144
Phosphofructokinase		37

ATP-dependent phosphorylation of both glucose (glucokinase) and fructose (fructokinase) to yield the corresponding hexose 6-P products. The activities of sucrose synthase, neutral invertase, glucokinase and fructokinase in whole cell extracts were similar.

Potential enzymes of UDPG metabolism were also assayed. The ADPG phosphorylase has been reported in a wide range of tissues (14), and a specific UDPG phosphorylase was recently identified in potato tubers (8). However, activities of ADPG and UDPG phosphorylase were essentially undetectable in sycamore cell extracts, both in the presence and absence of F26BP (Table I). Activity of nonspecific phosphodiesterase, which would convert UDPG to UMP plus glucose 1-P, was also undetected (data not shown). We were also unable to detect activity of ATP pyrophosphohydrolase (6), which hydrolyzes ATP to AMP + PPi, in crude extracts using a UDPG pyrophosphorylase coupled system. However, the cell extracts contained a high activity of UDPG pyrophosphorylase. A relatively low concentration of PPi (2 μM) supported an enzyme activity that was similar to that of sucrose synthase. Cell extracts also contained high activities (>200 nmol/mg protein·min) of phosphoglucoisomerase and phosphoglucomutase (data not shown).

Sycamore cell extracts contained two enzymes for conversion of fructose 6-P to fructose 1,6-bisP, *viz.* ATP-phosphofructokinase and PPi-linked phosphofructokinase (Table I). The latter activity was essentially dependent upon F26BP (Table I); in crude extracts, half-maximal activation required about 0.5 μM F26BP. Although whole cell extracts contain activity of fructose 1,6-bisphosphatase, this enzyme appears to be exclusively localized in the amyloplasts (T Teramae, M Nishimura, T Akazawa, unpublished data).

To obtain more information about some of the enzymes found in cell extracts, the enzymes were partially purified by ion exchange chromatography using a Pharmacia FPLC system with a Mono Q column.

Sucrose Synthase and Invertase. Figure 1 shows the elution profiles of invertase and sucrose synthase activities obtained by Mono Q chromatography of a whole cell extract. Invertase activity eluted at a slightly lower salt concentration compared with sucrose synthase, but complete separation was not achieved. The inset of Figure 1 shows that the invertase activity recovered from the column had a pH optimun close to pH 7.0; hence, the enzyme can be classified as a neutral invertase. The invertase activity was strongly inhibited by 50 mM Tris buffer (Fig. 1, inset), which is consistent with previous reports (12). Our standard invertase assays contained a low concentration of Tris (1–2.5 mM), that was introduced with the enzyme sample. Inhibition of the sycamore cell invertase by 2.5 mM Tris was less than 10%, as 15 to 20 mM Tris was required for 50% inhibition (data not shown). It was verified that desalting the enzyme into a Hepes buffer had no significant effect on observed activity or the K_m (sucrose). It is interesting that the sycamore invertase is considerably less sensitive to Tris inhibition compared with the soybean nodule enzyme, which required only 0.7 mM Tris for 50% inhibition (12).

The partially purified invertase and sucrose synthase preparations, obtained from ion exchange chromatography, were used to determine the K_m for sucrose. The apparent K_m for sucrose of sucrose synthase was about 15 mM, compared with 65 mM for invertase (Fig. 2). Similar values for K_m (sucrose) were obtained when crude extracts were used as the source of enzyme (data not shown).

UDPG Pyrophosphorylase. The UDPG pyrophosphorylase activity was eluted from the Mono Q column as a single peak of activity (Fig. 3). With the partially purified enzyme, the K_m for Mg·PPi was about 25 μM (Fig. 3, inset), which is similar to the value obtained using crude cell extracts (32 μM).

Hexose Kinase Activities. In preliminary experiments, there were indications that several hexose kinase activities were present in sycamore cell extracts. In particular, whole cell extracts contained substantial kinase activities with either glucose or fructose

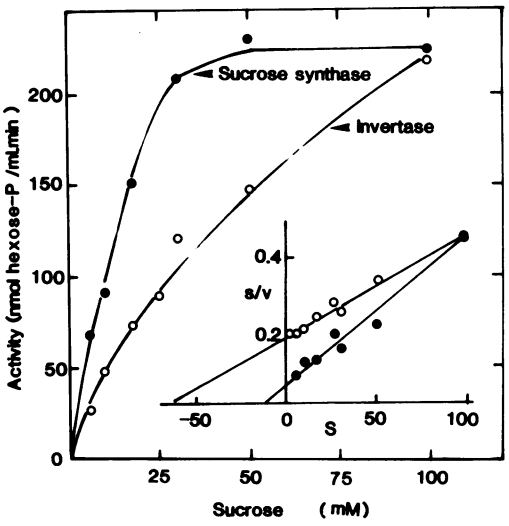

FIG. 2. Effect of sucrose concentration on the activity of partially purified invertase and sucrose synthase. Inset shows Woolf replots of the kinetic data.

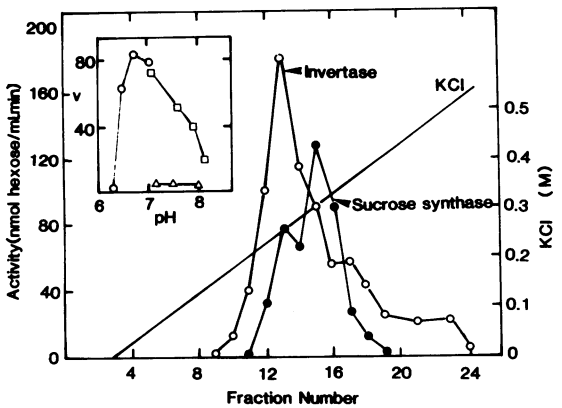

FIG. 1. Ion exchange chromatography of invertase and sucrose synthase from a crude sycamore cell extract on a Mono Q column. Inset shows the effect of pH on invertase activity (arbitrary units); buffers (50 mM) used were imidazole-HCl (O), Hepes-KOH (□), and Tris-HCl (Δ).

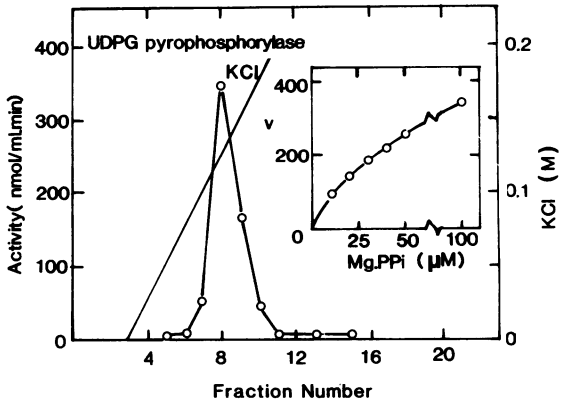

FIG. 3. Elution profile of UDPG pyrophosphorylase activity during ion exchange chromatography (Mono Q) of a crude sycamore cell extract. Inset shows PPi dependence of the peak fraction; the K_m (PPi) was calculated to be 25 μM from a Woolf replot of the data.

as substrate, whereas isolated amyloplasts only contained glucose kinase activity (data not shown). Another difference between the glucose and fructose kinase activities observed in whole cell extracts concerned apparent specificity for the trinucleotide. Kinase activity with glucose as substrate was reasonably specific for ATP, whereas kinase activity with fructose as substrate was remarkably nonspecific (Table II). When the various trinucleotides were tested at a concentration of 1 mM, the relative rates of phosphorylation of fructose were 100 (UTP), 88 (CTP), 76 (ATP), and 42 (GTP).

Ion exchange chromatography resolved two peaks of hexose kinase activity; the major peak eluted at a higher (0.30 M) salt concentration than the minor peak, which eluted at low salt (0.15 M) (Fig. 4). Both peaks contained kinase activity with fructose (Fig. 4A) and glucose (Fig. 4B) as substrate. The elution profiles for fructokinase activity with ATP or UTP as substrates were essentially identical, which suggests, but certainly does not prove, that the fructokinase activity observed with ATP and UTP was catalyzed by the same enzyme(s). The elution profiles of hexokinase and fructokinase were similar but not identical; the major peak (0.30 M) contained both activities but glucokinase activity eluted at a slightly lower salt concentration than did fructokinase. Thus, the enzymes were not resolved with the chromatographic conditions used.

Table II. *Nucleoside Triphosphate Specificity of Glucokinase and Fructokinase Activities in Desalted Cell Extracts of Sycamore*

Nucleoside-TriP (1 mM)	Activity	
	Glucokinase	Fructokinase
	nmol/mg protein·min	
ATP	11.4	14.1
GTP	1.0	7.8
UTP	2.7	18.6
CTP	2.5	16.3

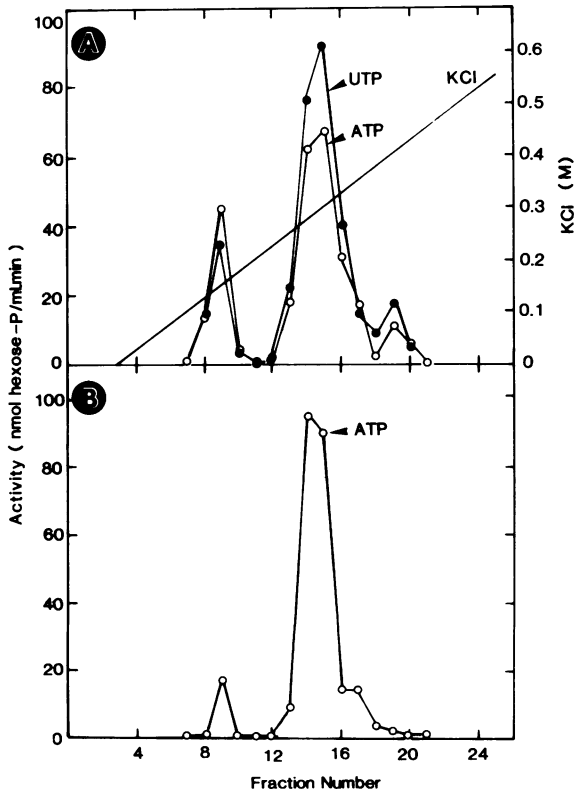

FIG. 4. Ion exchange chromatography of a crude sycamore cell extract on a Mono Q column. A, Fructokinase, assayed with ATP (○) or UTP (●); B, hexokinase, assayed with glucose and ATP.

The major peak of hexokinase activity, obtained from Mono Q chromatography, was studied further in terms of trinucleotide specificity. Glucokinase activity was extremely specific for ATP, whereas fructokinase activity was substantial with the four trinucleotides tested (Fig. 5). With fructokinase, the V with UTP was higher than that with ATP; however, the K_m (ATP) was about 5-fold lower than the K_m (UTP) (Table III). Consequently, the V/K_m ratios for ATP and UTP only differed by a factor of three. In contrast, with glucokinase, the K_m (ATP) was about 200-fold lower than the K_m (UTP), and the V/K_m ratios differed substantially (about 130-fold; Table III). Very similar kinetic constants were obtained for hexokinase and fructokinase activities in desalted cell-free extracts. However, in crude extracts, the K_m (UTP) of fructokinase was somewhat lower (0.30 mM), and the V/K_m ratios for ATP and UTP differed by a factor of two (data not shown). It is also worth noting that, using our assay system and commercially obtained yeast hexokinase, the fructokinase activity measured was extremely specific for ATP. It can be concluded that, in sycamore cells, hexose kinase activity with glucose as substrate is specific for ATP, whereas with fructose as substrate, *i.e.* fructokinase, UTP as well as ATP may be effective substrates.

PPi, ATP, and F26BP Concentration in Sycamore Cells. The endogenous amounts of PPi, ATP, and F26BP were measured in cell extracts. As expected, the cells contained a large ATP pool, but in addition, a PPi pool that was about 1/10 that of ATP (Table IV). The cells also contained endogenous F26BP; similar amounts, per g fresh weight, have been detected in a variety of plant tissues (9). The concentration of these metabolites on a molar basis cannot be accurately calculated because the volume

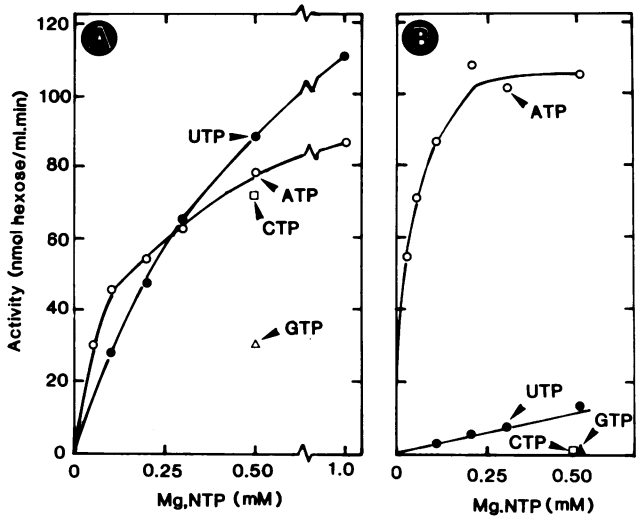

FIG. 5. Saturation kinetics for various nucleoside triphosphates (NTP) of partially purified (A) fructokinase and (B) hexokinase from sycamore cells.

Table III. *Kinetic Constants of Partially Purified Fructokinase and Glucokinase from Sycamore Cell Extracts*

Enzyme	Nucleoside·TriP	K_m	V	V/K_m
		mM	*nmol/mg protein·min*	*ratio*
Fructokinase	ATP	0.1	100	1000
	UTP	0.5	167	334
Glucokinase	ATP	0.03	120	4000
	UTP	6.0	180	31

Table IV. *Amounts of ATP, PPi, and F26BP in Cultured Sycamore Cells*

Metabolite	Concentration[a]	Recovery[b]
	nmol/g fresh wt	%
ATP	31.2 ± 1.5	80
PPi	3.2 ± 0.3	45
F26BP	0.09 ± 0.02	75

[a] Mean of three determinations ± SE; values are not corrected for recovery. [b] Average of two experiments.

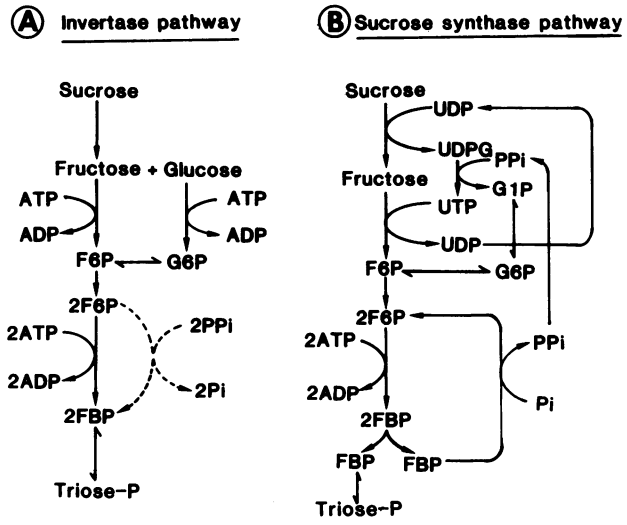

FIG. 6. Proposed pathways of sucrose degradation and glycolysis in sycamore cells. A, Invertase pathway; B, sucrose synthase pathway. See text for discussion.

of the space they are contained within the cell is not known. However, assuming that PPi and F26BP are confined to the cytosol, which comprises about 10% of the cell volume (1 ml/g fresh weight), the concentrations of PPi and F26BP would be 32 μM and 0.9 μM, respectively. The measured PPi concentration is an underestimate, because the recovery of PPi was only 45% (Table IV); the potential problems associated with extraction and recovery of the labile PPi molecule have been discussed previously (7), although higher recoveries have been reported by other investigators (7, 19).

DISCUSSION

Based on the results obtained in the present study, we postulate that two pathways may function in the degradation of sucrose in cultured sycamore cells (Fig. 6). In addition to the classical scheme which involves invertase (Fig. 6A), and was postulated by MacDonald and ap Rees (10) to function in cultured soybean cells, we propose the pathway shown in Figure 6B. The new pathway involves the initial metabolism of sucrose by sucrose synthase, followed by metabolism of the fructose and UDPG produced. The key features of this pathway are cycling of a) uridylates (UDP/UTP) and b) PPi. A central enzyme to the metabolite cycling is UDPG pyrophosphorylase, which we propose acts as an effective PPi-scavenger as a result of the high maximum activity of this enzyme (Table I) and a relatively low K_m (PPi) (Fig. 3). The UTP generated by the UDPG pyrophosphorylase reaction could be used to phosphorylate fructose in the UTP-linked fructokinase reaction, thereby forming the UDP required for sucrose synthase.

In order for UDPG pyrophosphorylase to operate as shown in Figure 6B, a source of PPi must exist. At the present time, PPi-linked phosphofructokinase is the only suitable enzyme that has been detected in sycamore cells. We propose that this enzymic reaction may function, at least under certain conditions, to generate PPi. This would mean that some 'futile cycling' of fructose 1,6-bisP/fructose 6-P occurs: for every two molecules of fructose 1,6-bisP produced by phosphofructokinase, one molecule would be converted back to fructose 6-P via PPi-linked phosphofructokinase.

The sucrose synthase pathway proposed requires that cells contain both PPi and F26BP. Both metabolites were detected in sycamore cells (Table IV), and the estimated molar concentrations in the cytosol are substantial: 32 μM PPi, which is close to the K_m (PPi) for UDPG pyrophosphorylase, and 0.9 μM F26BP, which is similar to the concentration required for half-maximal stimulation of PPi-linked phosphofructokinase in cell extracts. These results are consistent with the proposed pathway, but certainly do not identify either the source of the PPi pool or the enzyme(s) utilizing the PPi as substrate. For example, the reversible PPi-linked phosphofructokinase may either form or consume PPi. In higher plants, the enzyme is thought to be primarily involved in glycolysis (PPi consumption) (3), but in one case (*Propionibacteria shermanii*), the enzyme is thought to function in both glycolysis and glucogenesis (16). It is quite possible that PPi-linked phosphofructokinase may function in both directions, depending on conditions, in higher plants as well (9).

In sucrose-depletion experiments, Rebeille *et al.* (18) estimated the rate of endogenous sucrose degradation in sycamore cells to be 5 μmol of sucrose/g fresh weight·h. Assuming that cells contain about 6 mg soluble protein/g fresh weight, the rate of sucrose breakdown can be calculated to be about 14 nmol sucrose/mg protein·min, which is very similar to the measured activities of several enzymes in whole cell extracts (see Table I).

How sucrose is partitioned between the two pathways (Fig. 6) may be regulated primarily by the concentration of sucrose. Sucrose synthase (K_m, 15 mM) from sycamore cells has a much lower K_m for sucrose compared with the neutral invertase (K_m, 65 mM). Consequently, the sucrose synthase pathway may be relatively more important when sucrose availability is limiting. This pathway is also more energetically efficient, as the energy contained in the glycosidic linkage of the sucrose molecule is preserved. Thus, to metabolize one molecule of sucrose to the level of triose-P requires the input of three ATP in the sucrose synthase pathway, compared with four ATP in the invertase pathway. Consequently, it may be beneficial to the cells to have the most efficient pathway operate when carbon supplies are limiting. It is interesting to note that soybean nodules also contain both sucrose synthase and alkaline invertase, but the affinity for sucrose of the invertase is much higher than that of sucrose synthase (12, 13).

The concentration of F26BP in the cytosol may also regulate operation of the sucrose synthase pathway. By analogy to other systems, it is likely that F26BP concentration will fluctuate with changes in carbon supply, i.e., sucrose availability (for review, see Refs. 3 and 9). When the sucrose concentration is low, F26BP concentration would also be expected to be low. The PPi-linked phosphofructokinase exists in two interconvertible forms (22, 23); the small form favors gluconeogenesis (F6P production) whereas the large form favors the glycolytic direction (FBP production) (23). A high concentration of F26BP facilitates the aggregation of the enzyme from the small to large form (23). Hence, as F26BP concentration increases, production of PPi by PPi-linked phosphofructokinase may be reduced, thereby slowing carbon flow through the sucrose synthase pathway. Under these conditions, PPi-linked phosphofructokinase may function in the glycolytic direction and contribute to the invertase pathway.

It must be stressed that the sucrose synthase pathway proposed

is highly speculative and based solely on the occurrence and properties of certain enzymes. However, all of the necessary components of the pathway have been demonstrated. The proposed pathway is provided as a working model for future experiments, which will be necessary to determine whether these two pathways function *in vivo*. Future studies will be required to answer the many remaining questions concerning the operation of the proposed pathways, and aspects of their regulation.

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