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A Potential Pathway for Galactose Metabolism in *Cucumis sativus* L., A Stachyose Transporting Species[1]

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ABSTRACT

Conversion of [^{14}C]galactose (Gal) 1-P, UDP-[^{14}C]Gal, or UDP-[^{14}C]glucose to [^{14}C]sucrose was observed when cell-free homogenates of cucumber (*Cucumis sativus* L.) fruit peduncles were incubated with individual ^{14}C-labeled substrates, appropriate cofactors, and fructose. The sucrose product was labeled only in the glucose moiety. Conversion of [^{14}C]Gal-1-P to [^{14}C]sucrose was catalyzed by extracts of peduncles from all other stachyose transporting species tested, as well as green bean (a sucrose transporter) but was not catalyzed by peduncle extracts from three other sucrose transporting species. In cucumber, the ability of extracts to form [^{14}C]sucrose from [^{14}C]Gal-1-P was greater when peduncles were harvested from growing fruit than from unpollinated ovaries. [^{14}C]Sucrose formation from [^{14}C]Gal-1-P was inhibited by Mg•PPi, Mg•UDP, UMP, and sucrose. α-Galactosidase, galactokinase, UDP-gal pyrophosphorylase, UDP-Gal-4′-epimerase, UDP-glucose pyrophosphorylase, and sucrose synthase activities were detected in peduncle extracts. Neither sucrose phosphate synthetase nor hexose-1-P uridyltransferase were detected. Peduncle tissue contained a small pool of free galactose. These results suggest a potential pathway for the metabolism of galactose moieties hydrolyzed from stachyose, the major sugar transported by cucumber plants.

Stachyose (*O*-α-D-galactopyranosyl-(1→6)-*O*-α-D-galactopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside), a member of the raffinose family of oligosaccharides, is the major carbohydrate translocated by *Cucumis sativus* L. and other species (30, 31). Stachyose is also a major reserve carbohydrate in many seeds (1, 4, 24). The enzymes involved in raffinose saccharide biosynthesis are known, but the pathways of degradation of these sugars have not been studied extensively (5).

The initial step in stachyose and raffinose catabolism is thought to be hydrolysis by α-galactosidase (9, 18, 25, 28). However, the pathway by which the released gal moieties are metabolized in a stachyose utilizing tissue has only been suggested and not documented experimentally (1, 5, 16). The Leloir pathway is widely recognized as one route by which Gal may be converted to Glc-1-P (11, 12) and involves the sequential action of galactokinase, hexose-1-P uridyltransferase, and UDP-Gal-4′-epimerase. Other studies using Gal-adapted sugarcane suspension cultures (14) and *Bifidobacterium bifidum* (11) suggest a series of reactions involving UDP-Gal pyrophosphorylase, rather than hexose-1-P uridyltransferase, in which Gal can be converted to Glc-1-P for entry into central metabolism. In general, Gal metabolism and its regulation in higher plants is poorly understood.

Because cucumber fruit peduncles contain sucrose as their major soluble sugar (17), they apparently metabolize stachyose extensively. Thus, we have utilized cell-free extracts of this sink tissue to identify a sequence of reactions potentially involved in the metabolism of Gal moieties from stachyose.

MATERIALS AND METHODS

Plant Materials. Cucumber (*C. sativus* L. cv. Chipper) plants grown in a greenhouse were used for most studies. Pollinations were carried out by hand to assure the use of seeded fruit. Peduncles were harvested from plants with a single growing fruit and immediately placed at 4°C. In studies involving unpollinated ovaries, field grown plants were used; peduncles were harvested from ovaries with unopened flowers. Tomato (*Lycopersicon esculentum* Mill.), edible soybean (*Glycine max* Merr.), green pepper (*Capsicum annum* L.), green bean (*Phaseolus vulgaris* L.), butternut squash (*Cucurbita moschata* L.), cantaloupe (*Cucumis melo* L.), and watermelon (*Citrullus lanatus* Thunb.) plants were field grown. Fruit were harvested when approximately one-half full size. Fully expanded leaves were sampled from the same plants.

For extraction of SS[3] and SPS from cucumber leaf tissue, fully expanded leaves from the seventh node were harvested from plants which supported a rapidly growing fruit.

Freshly harvested tissue was used for all enzyme extractions. Assays were conducted on the same day the extracts were prepared.

Enzyme Extractions and Assays. Tissue was sliced into small sections and homogenized with a mortar and pestle in 5 volumes of the appropriate extraction buffer. The homogenate was then centrifuged at 27,000*g* for 15 min. Extraction procedures were carried out at 4°C.

α-Galactosidase was extracted in 100 mM Na-acetate (pH 5.2), containing 1 M NaCl. Activity was estimated by measuring the absorbance of *p*-nitrophenol hydrolyzed from *p*-nitrophenyl-α-D-galactopyranoside (17).

Galactokinase was extracted in 50 mM Tris-maleate (pH 7.4), containing 1 mM EDTA and 0.8 mM Gal. After centrifugation, the supernatant was desalted using a Sephadex G-25 column and assayed. The complete reaction mixture (100 μl) contained 25 mM Tris-maleate (pH 7.4), 5 mM $MgCl_2$ 1 mM ATP, 0.4 mM [^{14}C]Gal (2.3 μCi/μmol), 0.5 mM EDTA, and 50 μl extract. Reaction mixtures were incubated at 30°C and the reactions terminated by

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[2] This work is a portion of a thesis submitted by the first author in partial fulfillment for the PhD degree.

[3] Abbreviations: SS, sucrose synthase; SPS, sucrose phosphate synthetase; dH_2O, distilled water; gfw, grams fresh weight.

immersion in a boiling water bath for 90 s. Conversion of [^{14}C]Gal to a ^{14}C-labeled charged product was measured by spotting an aliquot containing the product on a 2.5 × 20 cm strip of DEAE-cellulose paper (Whatman DE 81). Papers were developed with dH_2O, dried at 40°C, and cut into 18-mm segments. Paper segments were placed into vials containing 15 ml of a standard toluene scintillation fluid (6 g/l PPO and 0.3 g/l POPOP) and the radioactivity assayed using a Packard Tri-carb scintillation counter. Radioactivity which remained at the origin (charged product) or which moved with the front (neutral substrate) was quantitated and the percent conversion of neutral substrate to charged product calculated.

Characterization of the galactokinase product involved incubating 50 μl of the charged product with 50 μl glycine-NaOH (pH 10.4), containing 0.65 unit of *Escherichia coli* phosphatase, at 37°C for 1 h. An aliquot of this mixture was then spotted on a DEAE-cellulose strip which was developed as described above. The neutral product formed during phosphatase treatment was eluted from the strip with dH_2O and identified using paper chromatography.

Sucrose synthase and SPS were extracted in 20 mM Hepes-NaOH (pH 7.5), containing 1% BSA, 1 mM EDTA, 5 mM DTT, and 0.5% PVP-10. After centrifugation, the supernatant was desalted using a Sephadex G-25 column and assayed for SS and SPS activity by measuring the Fru or Fru-6-P-dependent formation of UDP from UDP-Glc using the procedure of Salerno and Pontis (20).

Hexose-1-P uridyltransferase and UDP-Gal-4′-epimerase were extracted using the same procedure as for preparing desalted extracts for sucrose formation from ^{14}C-labeled substrates. The extract was assayed for hexose-1-P uridyltransferase activity according to a procedure previously described (8). UDP-Gal-4′-epimerase was assayed spectrophotometrically by measuring the amount of UDP-Glc formed from UDP-Gal. UDP-Glc was assayed by measuring the production of NADH during the oxidation of UDP-Glc by UDP-Glc dehydrogenase (15).

Sucrose Formation From ^{14}C-Labeled Substrates. Peduncle tissue was homogenized using a chilled mortar in 5 volumes of 50 mM Hepes-NaOH (pH 7.2), containing 1 mM EDTA, 0.5 mM DTT, and 0.5% (v/v) Triton X-100. The homogenate was centrifuged at 27,000*g* for 15 min. Solid ammonium sulfate was added to the supernatant and the protein insoluble between 0.3 and 0.7 saturation was collected by centrifugation and dissolved in 50 mM Hepes-NaOH (pH 7.2), containing 1 mM EDTA and 0.5 mM DTT. The extract was desalted using a Sephadex G-25 column.

Sucrose was formed by incubating 50 μl desalted extract and appropriate cofactors with either Gal-1-P, UDP-Gal, or UDP-Glc. Reaction mixtures (100 μl) contained 25 mM Hepes-NaOH (pH 7.2), 0.5 mM EDTA, 0.25 mM DTT, 10 mM Fru, and either 25 μM [^{14}C]Gal-1-P (13.6 μCi/μmol), 25 μM UDP-[^{14}C]Gal (4.5 μCi/μmol), or 25 μM UDP-[^{14}C]Glc (4.6 μCi/μmol). Reactions with Gal-1-P also contained 1 mM UTP and 6 mM $MgCl_2$. Reactions were carried out at 30°C and were terminated by adding 400 μl 100% ethanol. Negatively charged compounds were removed by adding 200 μl of a stirred slurry of Dowex 1-X8 (formate form) anion exchange resin (suspended 1:1, v/v, in dH_2O) and incubating at 25°C for 20 min with constant shaking. Samples were centrifuged for 1 min to pellet the resin and a 300-μl aliquot removed and placed in a vial containing 15 ml of a standard toluene scintillation fluid containing 33% (v/v) Triton X-100. Radioactivity was assayed and the Fru dependent conversion of charged substrate to neutral product was calculated.

For product characterization, 10 μl 40 mM acetic acid were added to 100 μl of each reaction product to adjust the pH to 4.6. Then, 100 units of yeast invertase were added and the samples incubated for 1 h at 55°C. Reactions were terminated by immersion in a boiling water bath for 90 s. ^{14}C-Labeled sugars were then identified by paper chromatography.

Extraction, Identification, and Assay of Soluble Sugars. Procedures used for ethanolic extraction of soluble sugars and their identification using paper chromatography were previously described (17). To specifically assay for Gal, 1 ml of an 80% ethanol extract was applied to a column containing 1-ml layers of Dowex 1-X8 and Dowex 50 exchange resins (26) and the column eluted with 5 ml 80% ethanol. The eluate was evaporated to dryness *in vacuo* at 40°C and the residue dissolved in 300 mM Tris-HCl (pH 8.6), containing 4 mM GSH and 0.4 mM NAD^+.

Gal present in the samples was calculated from the amount of NADH formed after the oxidation of Gal to galactonic acid by Gal dehydrogenase (6).

Paper Chromatography. Reaction products were spotted on Whatman No. 1 chromatography paper for separation of neutral sugars. Papers were developed in butanol:benzene:pyridine:water (5:1:3:3) for 40 h and sugars visualized as previously described (17). Chromatograms of ^{14}C-labeled products were cut into 5-mm segments, placed into vials containing 15 ml of a standard toluene scintillation fluid, and the radioactivity assayed.

Protein Determination. The amount of protein in plant extracts was estimated using the procedure of Lowry *et al.* (13); casein was used as the standard.

RESULTS

Occurrence of Free Galactose and α-Galactosidase in Cucumber Fruit Peduncles. By using Gal dehydrogenase as a specific and sensitive assay, a small pool of 37.7 ± 8.5 μg Gal/gfw was detected in cucumber peduncle tissue. α-Galactosidase activity was also detected and ranged from 141 to 178 nmol of product/min·gfw for two independent samples of peduncles.

Detection of Galactokinase Activity. Preliminary attempts to demonstrate the presence of galactokinase in peduncle extracts were unsuccessful. However, the addition of 0.8 mM gal to the extraction buffer had a marked stabilizing effect on the enzyme and galactokinase activity was subsequently detected. Similar to galactokinase from other sources (3, 21, 23), the reaction was dependent on $MgCl_2$ and specific for ATP as phosphate donor (Table I). When treated with *E. coli* phosphatase, the charged product of the reaction was completely labile. The neutral product formed during phosphatase treatment co-chromatographed with authentic Gal on paper chromatograms (data not shown).

Formation of [^{14}C]Sucrose from ^{14}C-Labeled Substrates by Cucumber Peduncle Extracts. When desalted cucumber peduncle extracts were incubated with Fru, appropriate cofactors, and either

Table I. *Nucleotide Triphosphate Specificity and Mg^{2+} Dependence of the Galactokinase Reaction in Crude Extracts from Cucumber Fruit Peduncles*

Reaction Condition[a]	Amount Charged Product Formed after 30 min
	nmol
(−) Nucleotide triphosphate	0.40
(+) 1 mM ATP[b] (complete reaction)	18.88
(+) 1 mM UTP	0.88
(+) 1 mM CTP	0.72
(+) 1 mM GTP	0.40
Complete (−) 5 mM $MgCl_2$	0.40
Boiled enzyme control	0.32

[a] Amendments in complete reactions are described under "Materials and Methods." There were 40 nmol of [^{14}C]Gal present initially.

[b] The charged product of the complete reaction was 100% phosphatase labile. The neutral product resulting from phosphatase treatment cochromatographed with authentic galactose using descending paper chromatography.

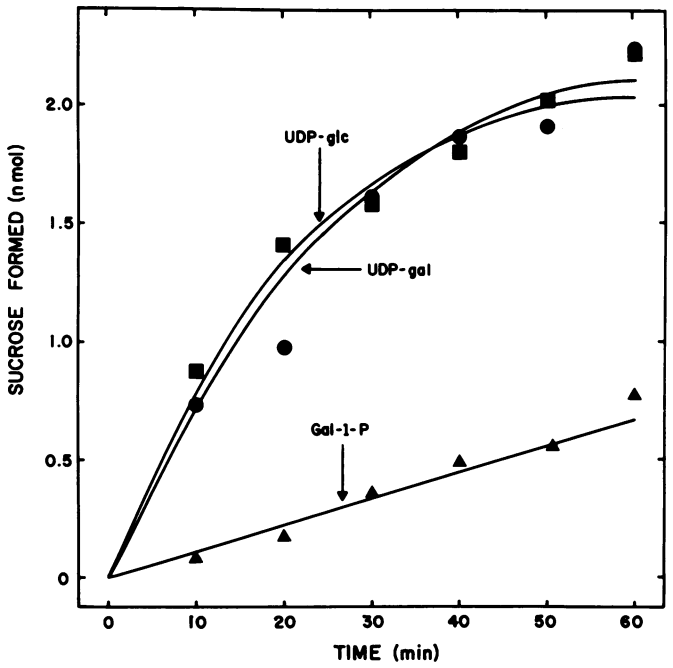

FIG. 1. The formation of [^{14}C]sucrose during incubation of cucumber fruit peduncle extract with [^{14}C]Gal-1-P, UDP-[^{14}C]Gal, or UDP-[^{14}C]Glc.

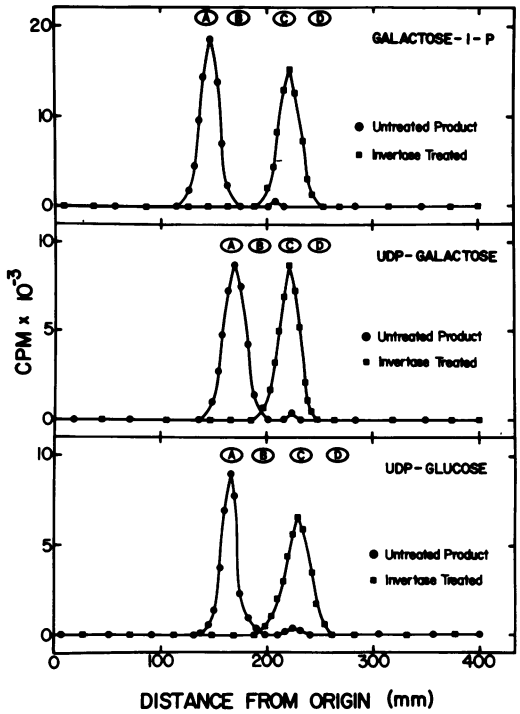

FIG. 2. Graphs of radiochromatograms of the untreated (●) and the invertase treated (■) ^{14}C-labeled product formed during incubation of cucumber fruit peduncle extract with either [^{14}C]Gal-1-P (top), UDP-[^{14}C]Gal (center), or UDP-[^{14}C]Glc (bottom). Sugar standard spots: A, sucrose; B, galactose; C, glucose; D, fructose.

[^{14}C]Gal-1-P, UDP-[^{14}C]Gal, or UDP-[^{14}C]Glc, a ^{14}C-labeled product was formed which was subsequently shown to be sucrose. The rate of [^{14}C]sucrose formation was greater from UDP-[^{14}C]Gal and UDP-[^{14}C]Glc than from [^{14}C]Gal-1-P (Fig. 1). The products from the reactions had chromatographic mobilities identical to sucrose (Fig. 2). No other major neutral products were detected on chromatograms. Treatment of the reaction products with invertase revealed that the ^{14}C was present exclusively in the Glc moiety of the [^{14}C]sucrose (Fig. 2). Formation of [^{14}C]sucrose from the three ^{14}C-labeled substrates was dependent on the addition of Fru to the reaction mixture (Table II). [^{14}C]Sucrose formation required $MgCl_2$ and UTP when [^{14}C]Gal-1-P was used as the substrate. When reactions containing Fru-6-P rather than Fru were incubated, [^{14}C]sucrose formation was not observed. [^{14}C]Sucrose-P also was not formed since treatment of the terminated reactions with *E. coli* phosphatase did not reveal the presence of a product (data not shown).

Table II. *The Dependence of [^{14}C]Sucrose Formation on Fructose and the Mg^{2+} and UTP Dependence with [^{14}C]Gal-1-P as substrate*

Substrate	Reaction Condition[a]	% Conversion[b]
[^{14}C]Gal-1-P	Complete	32.9
	Boiled enzyme	2.3
	(−) $MgCl_2$	1.7
	(−) UTP	2.5
	(−) Fructose	2.0
UDP-[^{14}C]Gal	Complete	95.5
	Boiled enzyme	1.8
	(−) Fructose	9.4
UDP-[^{14}C]Glc	Complete	89.8
	Boiled enzyme	1.3
	(−) Fructose	8.5

[a] Amendments in complete reactions are described under "Materials and Methods."

[b] There was 2.5 nmol of substrate present initially. Reactions were terminated after 30 min.

Table III. *The Activity of Sucrose Synthase and Sucrose Phosphate Synthase in Cucumber Leaves and Peduncles*

Product formation was linear with time and proportional to the amount of enzyme extract added to the reaction. Data represent the means from three separate experiments.

Tissue	Activity	
	Sucrose Synthase	Sucrose phosphate synthase
	(*nmol/min·gfw*) ± SE	
Peduncles	280.6 ± 103.1	0
Leaves	135.5 ± 71.7	271.1 ± 87.7

Enzyme Activities. The above results suggested that the conversion of Gal-1-P to sucrose occurred through the sequential action of UDP-Gal pyrophosphorylase, UDP-Gal-4′-epimerase, and SS. Spectrophotometric assays for these enzymes revealed their presence in crude extracts of fruit peduncle tissue. UDP-Gal pyrophosphorylase and UDP-Glc pyrophosphorylase were detected in cucumber peduncle extracts (data not shown). A further study of these enzymes was undertaken and a manuscript is in preparation (E. L. Smart, personal communication). UDP-gal-4′-epimerase activity was present, based on the results of a single experiment, at 1.31 μmol of product/min·gfw.

Sucrose synthase and SPS activities were compared in cucumber peduncles and in mature, fully-expanded leaves. Sucrose phosphate synthetase activity was not detected in peduncle extracts (Table III). Leaf extracts contained both enzyme activities, whereas peduncle extracts contained only SS activity.

Hexose-1-P uridyltransferase activity was not detected in two separate, freshly-prepared peduncle extracts.

Capacity of Extracts of Fruit Peduncles from Various Species to Catalyze the Conversion of [^{14}C]Gal-1-P to [^{14}C]Sucrose. In Table IV, data are presented which confirm the general occurrence of Gal-containing oligosaccharides in cucurbits. Extracts of fruit peduncles from all species which contained raffinose saccharides

Table IV. *Soluble Sugars Present in Leaves and Fruit of Various Species and the Rate of [^{14}C]Sucrose Formation from [^{14}C]Gal-1-P by Extracts of Their Fruit Peduncles*

An aliquot containing 200 μg of total carbohydrate from each sample was spotted. Five μg was the minimal detectable amount for each sugar. All sugars were easily detectable except for St and Raf in the fruit of *Cucurbita moschata* which were barely detectable. [^{14}C]Sucrose synthesis assays were conducted the same as for cucumber extracts as described under "Materials and Methods." In all cases, [^{14}C]sucrose formation was dependent on fructose, linear with time, and proportional to the amount of enzyme added. [^{14}C]Sucrose was the only sugar detected on chromatograms. Only the glucose moiety was ^{14}C-labeled. Abbreviations: St, stachyose; Raf, raffinose; Vr, verbascose; Su, sucrose; Glc, glucose; Fru, fructose.

Species	Sugars Detected on Chromatograms of Ethanolic Extracts		Rate of Sucrose Formation	
	Leaves	Fruit	Per gfw	Per mg protein
			nmol/min	
Citrullus lanatus Thunb.	St, Raf, Su, Glc, Fru	Su, Glc, Fru	3.7	1.0
Cucurbita moschata L.	Vr, St, Raf, Su, Glc, Fru	St, Raf, Su, Glc, Fru	67.2	17.7
Cucumis melo L.	Raf, Su, Glc, Fru	Su, Glc, Fru	9.7	4.4
Capsicum annum L.	Su, Glc, Fru	Su, Glc, Fru	NP[a]	NP
Phaseolus vulgaris L.	Su, Glc, Fru	Su, Glc, Fru	9.6	0.7
Glycine max L.	Su, Glc, Fru	Su, Glc, Fru	NP	NP
Lycopersicon esculentum Mill.	Su, Glc, Fru	Su, Glc, Fru	NP	NP

[a] No product detected.

Table V. *Rate of [^{14}C]Sucrose Formation from [^{14}C]Gal-1-P by Extracts of Cucumber Peduncles from Unpollinated Ovaries and Growing Fruit*

Peduncles from[a]	Rate of [^{14}C]Sucrose Synthesis[b]		
	nmol/min·g fw	*nmol/min·mg protein*	*nmol/min·peduncle*
Unpollinated ovaries	4.1 ± 1.0	1.1 ± 0.4	0.13 ± 0.06
Growing fruit	7.7 ± 1.8	3.5 ± 0.4	1.20 ± 0.14

[a] The average fresh weight of peduncles from unpollinated ovaries and growing fruit was 29 and 178 mg, respectively.

[b] Activity is expressed as the mean ± SE of three replicate experiments. Product formation was dependent on fructose, linear with time, and proportional to the amount of enzyme extract added to the reaction mixture.

in leaf tissue were able to catalyze the conversion of [^{14}C]Gal-1-P to [^{14}C]sucrose. However, the ability to catalyze this conversion was not unique to peduncles from plants whose leaves contain detectable amounts of raffinose saccharide. Green bean, which contained no detectable raffinose saccharides, also catalyzed the conversion (Table IV).

Capacity of Crude Extracts of Cucumber Peduncles from Unpollinated Ovaries or Growing Fruit to Convert [^{14}C]Gal-1-P to [^{14}C]Sucrose. Utilizing short-time assays, it was found that the rate of conversion of [^{14}C]Gal-1-P to [^{14}C]sucrose was linear with time and proportional to the amount of enzyme extract added to the reactions (data not shown). This provided a means to measure the rate at which different extracts could form [^{14}C]sucrose from [^{14}C]Gal-1-P. This conversion by extracts was greater when peduncles were harvested from growing fruit than from unpollinated ovaries (Table V).

Inhibition of the Conversion of [^{14}C]Gal-1-P to [^{14}C]Sucrose. The conversion of [^{14}C]Gal-1-P to [^{14}C]sucrose by desalted extracts of cucumber peduncles from growing fruit was inhibited 38, 90, 55, and 15% by 0.1 mM Mg·PPi, 1 mM Mg·UDP, 1 mM UMP, and 100 mM sucrose, respectively (data not shown). Uridine and Pi were not inhibitory when tested at the same concentrations as Mg·UDP and Mg·PPi, respectively.

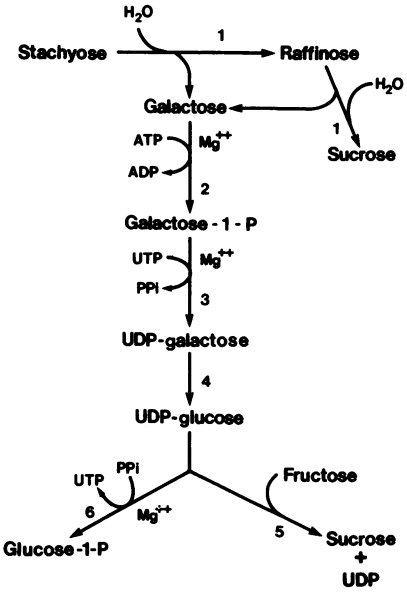

FIG. 3. Proposed pathway for stachyose catabolism to Glc-1-P or sucrose in cucumber fruit peduncle tissue. Enzymes: 1, α-galactosidase; 2, galactokinase; 3, UDP-Gal pyrophosphorylase; 4, UDP-gal-4′-epimerase; 5, sucrose synthase; 6, UDP-Glc pyrophosphorylase.

DISCUSSION

The results of this study suggest a pathway by which the Gal moieties, hydrolyzed from stachyose, may be converted to sucrose in cucumber peduncle tissue (Fig. 3). Other plant tissues convert supplied Gal primarily to sucrose, *in situ* (1, 10). The presence of these reactions in cucumber peduncles correlates well with some known facts about this species. Cucumber plants transport stachyose (30), yet sucrose, Glc, and Fru are the major free sugars detectable chromatographically in fruit (17). Sucrose is the predominant soluble sugar in peduncles, although stachyose and raffinose also are detectable (17).

A free Gal pool has not been detected in paper chromatographic

studies of soluble sugars in some raffinose saccharide utilizing tissues (9, 16, 17). As shown here, cucumber peduncles contain free Gal, but at a concentration below detection on paper chromatograms (9). The source(s) of this free Gal pool is unknown. One possibility is that it results from the enzymic hydrolysis of Gal moieties from stachyose and raffinose *in vivo*. It has been shown that cucumber α-galactosidases are capable of hydrolyzing the galactosidic linkages of stachyose, raffinose, and melibiose (9, 25). Also, cucumber callus cells contained a pool of Gal in their free space only when cultured on media containing raffinose saccharides as a carbon source (9). The Gal pool in cucumber peduncle tissue may be kept low through phosphorylation by galactokinase (Table I).

Hexose-1-P uridyltransferase, the central enzyme in the Leloir pathway of Gal metabolism (11, 12), was not detected in peduncle extracts. However, UDP-Gal pyrophosphorylase activity was present. Pyrophosphorylase pathways for Gal metabolism have been proposed elsewhere (2, 11, 14, 29). UDP-Gal pyrophosphorylase, which usually is associated with UDP-Glc pyrophosphorylase activity, has not been widely studied in plants although it has been detected in some plant extracts (7, 14, 16). Cucumber peduncles contain two UDP-Glc pyrophosphorylases, one of which is devoid of UDP-Gal pyrophosphorylase activity (E. L. Smart, personal communication). Thus, not all plant tissues which contain UDP-Glc pyrophosphorylase necessarily contain UDP-Gal pyrophosphorylase activity.

Extracts of fruit peduncles from all species which contained raffinose saccharides in ethanolic leaf extracts possessed the enzymic capacity to convert Gal-1-P to sucrose *in vitro* (Table IV). Three species which did not contain raffinose saccharides in leaf extracts did not catalyze this conversion. The fact that peduncle extract from green bean catalyzed this conversion implies the probable existence of UDP-Gal pyrophosphorylase in a species known to transport sucrose (30) and here found to be devoid of raffinose saccharides in leaf or fruit ethanolic extracts.

It has been postulated that hydrolysis of translocated carbohydrate is an integral part of sugar accumulation in *Zea mays* (22) and *Saccharum* sp. (19). In cucumber, extensive metabolism of stachyose occurs in fruit peduncles (17). However, the importance of this metabolism to sugar import into fruit has not been established. Low concentrations of raffinose saccharides were present in *Cucurbita moschata* L. fruit (Table IV). Thus, excluding the resynthesis of these sugars in the fruit, it seems unlikely that conversion of Gal-containing transport sugars to sucrose is a prerequisite to carbohydrate import into fruit of all cucurbits. However, sucrose is apparently the major sugar imported into cucumber fruit (17). Thus, increased metabolism of the Gal moieties of transported stachyose to sucrose might be expected prior to or during rapid fruit growth. The fact that the capacity of cucumber peduncle extracts to convert Gal-1-P to sucrose was greater when peduncles were harvested from growing fruit than from unpollinated ovaries (Table V) supports this contention. It is important to point out that the source of the free Fru needed for the sucrose synthase reaction is unknown. However, there is a pool(s) of Fru in cucumber peduncle tissue (17).

The physiological role of SS is often considered to be that of sucrose degradation, although the reaction is reversible and a synthetic role has not been ruled out (5, 27). The sequential action of SPS and sucrose phosphate phosphatase is more frequently considered to be responsible for sucrose synthesis (5). However, in cell-free peduncle extracts, SS acts in a synthetic fashion. Sucrose phosphate synthetase was not detected in peduncle extracts (Table III). Whether SS acts synthetically *in vivo* in cucumber peduncles is unknown. To address this, we have detected and partially characterized two forms of SS from peduncles (data not shown). Also, pulse-chase experiments using specifically labeled [^{14}C]stachyose (not commercially available) may further clarify the pathways of stachyose catabolism in this tissue, particularly the source of Fru needed for the sucrose synthase reaction.

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