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The Biosynthesis of Sucrose and Nucleoside Diphosphate Glucoses in *Phaseolus aureus*[1]

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ABSTRACT

Sucrose-phosphate synthetase is detectable only in intact chloroplast preparations of *Phaseolus aureus*. In contrast, sucrose synthetase and uridine diphosphate glucose (UDP-glucose) pyrophosphorylase activities are low in extracts of photosynthetic tissues of *P. aureus* but are high in extracts of nonphotosynthetic tissues. Activities for ADP-, dTDP-, CDP-, and GDP-glucose pyrophosphorylases are generally higher in extracts of photosynthetic tissues of *P. aureus* than in extracts of nonphotosynthetic tissues. The high levels of sucrose synthetase and of UDP-glucose pyrophosphorylase found in dark-grown hypocotyls begin to decline about 4 hours after exposure to light at a rate of 50% every 3 hours.

The data suggest that sucrose-phosphate synthetase and sucrose phosphatase are the enzymes responsible for the biosynthesis of sucrose from photosynthetically fixed CO_2, and that the major function of sucrose synthetase is to catalyze the synthesis of UDP-, ADP-, dTDP-, CDP-, and GDP-glucose from translocated sucrose in nonphotosynthetic tissues; in photosynthetic tissues the pyrophosphorylases may replace sucrose synthetase in catalyzing the synthesis of these nucleoside diphosphate glucoses. We offer the suggestion that sucrose synthetase and UDP-glucose pyrophosphorylase play a major role in the uptake and metabolism of sucrose in nonphotosynthetic tissues. Results are presented from preliminary studies on the conversion *in vitro* of sucrose to glucose 1-phosphate by the coupled reactions of sucrose synthetase and UDP-glucose pyrophosphorylase with highly purified preparations of these enzymes.

The following alternate mechanisms for the biosynthesis of sucrose and nucleoside diphosphate glucose have been demonstrated in plants:

$$\text{UDP-glucose} + \text{fructose-6-P} \xrightarrow{\text{sucrose-P synthetase}} \text{sucrose-P} + \text{UDP} \quad (1a)$$

$$\text{Sucrose-P} \xrightarrow{\text{sucrose phosphatase}} \text{sucrose} + P_i \quad (1b)$$

$$\text{NDP-glucose} + \text{fructose} \xrightleftharpoons{\text{sucrose synthetase}} \text{sucrose} + \text{NDP} \quad (2)$$

$$\text{Glucose-1-P} + \text{NTP} \xrightleftharpoons{\text{NDP-glucose pyrophosphorylase}} \text{NDP-glucose} + \text{pyrophosphate} \quad (3)$$

where N = uridine, adenosine, thymidine, cytidine, or guanosine.

The equilibrium constant of reaction 1a in the direction of sucrose-P synthesis has been reported to be 3250 at 38 C and pH 7.5 (11). When this reaction is coupled with sucrose phosphatase, the biosynthesis of sucrose can be considered essentially irreversible. Sucrose-P synthetase has been demonstrated in a variety of plants (3, 12, 14, 17) and has been suggested to be the enzyme responsible for the biosynthesis of sucrose from photosynthetically fixed CO_2.

In contrast, the reaction catalyzed by sucrose synthetase is freely reversible. This enzyme has also been demonstrated in a variety of plants (4, 10, 15, 16, 18). Grimes *et al.* (10) have partially purified and characterized sucrose synthetase from extracts of etiolated *Phaseolus aureus* seedlings. This enzyme can function with either UDP-, ADP-, dTDP-, CDP-, or GDP-glucose as substrate. The K*m* for UDP-glucose is 10-fold lower than for any of the other nucleoside diphosphate sugars, although all five nucleoside diphosphate glucoses are efficient substrates.

The pyrophosphorylase reactions are also freely reversible. In contrast to sucrose synthetase, where a single enzyme can catalyze the biosynthesis of five different nucleoside diphosphate glucoses, there apparently exists a separate pyrophosphorylase specific for each nucleoside diphosphate glucose. Pyrophosphorylases specific for UDP-, ADP-, and GDP-glucose have previously been reported in plants (2, 7, 9).

We have been examining the relationship among these enzymes in the mung bean *P. aureus* in an attempt to assess the relative physiological significance of each enzyme with regard to the biosynthesis of these compounds.

MATERIALS

Chemicals. ^{14}C-Fructose-6-P, ^{14}C-glucose-1-P, and UTP were obtained from Calbiochem; ^{14}C-fructose and ^{14}C-sucrose from Schwarz Biochemicals; ATP, dTTP, GTP, CTP, UDP-, ADP-, dTDP-, CDP-, and GDP-glucose from Sigma; DMSO[3] from J. T. Baker and Co.; and DE-52, a preswollen microgranular DEAE-cellulose, from Whatman.

METHODS

Growth of P. aureus. Dried *P. aureus* beans, obtained locally, were soaked 10 min in 10% Clorox, washed with distilled H_2O, and germinated for 24 hr in distilled H_2O with aeration. The

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[3] Abbreviations: DMSO: dimethylsulfoxide; DEAE-: diethylaminoethyl.

germinated seeds were planted in vermiculite moistened with half-strength Hoagland's solution. Etiolated seedlings were grown for 4 days in the dark at 23 C. Light-grown bean seedlings were grown for 7 to 10 days in a Percival growth chamber illuminated by incandescent and fluorescent light, with cycles of 14-hr days at 25 C and 10-hr nights at 16 C.

Preparation of Chloroplasts. Twenty grams of leaves from 7- to 10-day-old light-grown plants were homogenized for 15 sec in a Waring Blendor at 4 C with 40 ml of 20 mM tris-HCl, pH 7.3, containing 0.33 M sorbitol and 10 μM EDTA. The homogenate was filtered through cheesecloth and centrifuged for 1 min at 1400*g*. The pellet was washed twice with 7.5 ml of the extraction buffer which had been adjusted to 10% DMSO (v/v). This washing procedure removed all detectable traces of a soluble phosphatase which hydrolyzed fructose-6-P. The washed pellet was resuspended in 0.3 ml of the washing buffer and used for assay of sucrose-P synthetase. This preparation has been designated as "intact" chloroplasts on the basis of microscopic observation which showed a field of chlorophyllous organelles of homogeneous size.

Preparation of Sucrose Synthetase and of the Pyrophosphorylases. For the study of the distribution of enzyme activity in various tissues, the following procedure was used. All manipulations were performed in the cold. For etiolated seedlings, 5.0 g of hypocotyls or roots were ground in a chilled mortar with 5.0 ml of 30 mM tris-HCl, pH 7.5, containing 0.1 mM dithiothreitol and 10 μM EDTA. The homogenate was filtered through cheesecloth and centrifuged at 39,000*g* for 20 min. Powdered ammonium sulfate was added slowly until the solution reached 70% of saturation. The resulting suspension was stirred for 10 min and then centrifuged for 10 min at 39,000*g*. The pellet was resuspended in 0.5 ml of 10 mM tris-HCl, pH 7.5, containing 10 μM dithiothreitol and 10 μM EDTA. This solution was desalted by passage through a G-25 Sephadex column (1 × 15 cm) that had been pre-equilibrated in the resuspending buffer. The excluded volume was collected and used as a source of all enzymes. For light-grown tissues, the same procedure was employed. However, immediately after homogenization and before precipitation with ammonium sulfate, the crude extract was quickly passed through a 2.3- × 25-cm G-25 Sephadex column that had been pre-equilibrated with the extraction buffer. The excluded volume was collected. This procedure prevented browning of the extract.

Partial Purification of Sucrose Synthetase. A crude extract from 1 kg of 4-day-old etiolated seedlings was prepared as described above. Powdered ammonium sulfate was added until the solution reached 40% of saturation. The resulting suspension was stirred for 10 min and then centrifuged for 10 min at 39,000*g*. The pellet was discarded, and the supernatant solution was brought to 50% saturation by the further addition of ammonium sulfate. The pellet resulting from this procedure was desalted on a G-25 Sephadex column (2.3 × 30 cm) that had been pre-equilibrated with 50 mM sodium phosphate, pH 7.5, containing 10 μM dithiothreitol and 10 μM EDTA. The excluded volume was collected and applied directly to a column (1.5 × 25 cm) of Whatman DE-52 ion exchange resin that had been pre-equilibrated in the same buffer. The DE-52 column containing the enzyme was washed with 100 ml of the same buffer. The enzyme was then eluted with 300 ml of a 0 to 350 mM linear NaCl gradient prepared in the equilibration buffer. The fractions of peak activity were collected and concentrated by pressure dialysis at 50 p.s.i. in an Amicon Diaflo apparatus equipped with a UM-20E filter. The concentrated enzyme solution from the DE-52 column was passed through a 15 M Agarose column (1.4 × 89 cm) that had been pre-equilibrated in 10 mM cacodylate buffer, pH 7.3, containing 10 μM dithiothreitol and 10 μM EDTA. The peak fractions were pooled and used as enzyme for the experiment of Table VI. This preparation of sucrose synthetase was approximately 100-fold purified and was free of detectable invertase, phosphatase, and pyrophosphorylase.

Purification of UDP-glucose Pyrophosphorylase. The supernatant solution, which was 50% of saturation with respect to ammonium sulfate, prepared during the purification of sucrose synthetase, was brought to 65% of saturation by the further addition of ammonium sulfate. The resulting pellet was desalted and chromatographed on a DE-52 column by the identical procedure described for the purification of sucrose synthetase. The peak fractions from this column were pooled, concentrated, and applied to a 1.5 M Agarose column (2 × 100 cm) that had been pre-equilibrated with 10 mM tris-HCl, pH 7.5, containing 50 μM dithiothreitol and 10 μM EDTA. The peak fractions from this column were pooled and used for the experiment in Table VI. The enzyme was 256-fold purified and was free of detectable invertase, phosphatase, and pyrophosphatase.

Assay of Sucrose-P Synthetase. Twenty microliters of chloroplast preparation containing 1 to 2 mg/ml of chlorophyll were incubated 1 hr at 25 C with 0.1 μmole of UDP-glucose in a final volume of 40 μl. Reaction rates were linear over this time period and were proportional to chlorophyll concentration. The reactions were terminated by spotting them on Whatman No. 1 paper. When accurate timing was critical, the reactions were terminated by adding 5 μl of 0.1 M sodium tetraborate, pH 9.6, to the reaction mixture before spotting on the paper. The reaction mixtures were then electrophoresed (40 v/cm) in 50 mM sodium tetraborate, pH 9.6, for 75 to 90 min. Sucrose was detected and quantitated by the method of Grimes *et al.* (10).

Sucrose Synthetase. This enzyme was assayed by the method of Grimes *et al.* (10).

Pyrophosphorylases. These were assayed by a modification of the method of Barber and Hassid (2). Ten microliters of enzyme were incubated at 25 C with 5 nmoles of ^{14}C-glucose-1-P (3780 cpm/nmole), 0.25 μmole of $MgCl_2$, and 0.10 μmole of the appropriate nucleoside triphosphate (UTP, ATP, dTTP, GTP, or CTP) in a final volume of 20 μl. All pyrophosphorylases except UDP-glucose pyrophosphorylase were incubated 1 hr. Extracts were assayed for the particularly active UDP-glucose pyrophosphorylase by appropriate dilution and incubation for only 5 min. All reactions were linear with time for the period incubated. The reactions were terminated by pipetting them into 0.9 ml of 0.1 M ammonium sulfate, pH 5.0, containing 10 mM unlabeled glucose and glucose-1-P, and then by placing the diluted reactions in a boiling water bath for 1 min. After the tubes cooled, 0.1 ml of a 10%, w/v, suspension of Norit A was added. The tubes were allowed to stand 5 min with periodic shaking. They were then filtered onto 2-cm Whatman No. 1 filter paper discs. The discs were washed with 15 ml of distilled H_2O, then with 5 ml of 10% ethanol, and, finally, with 5 ml of 50 mM sodium tetraborate, pH 9.6. The discs, containing adsorbed nucleoside diphosphate glucoses, were dried and counted in a low background gas flow planchet counter.

Assay of Coupled Reaction (Table VI). Fifteen microliters of partially purified sucrose synthetase plus 5 μl of partially purified UDP-glucose pyrophosphorylase (approximate ratio of activities 1:15) were incubated for 1 hr at 25 C in 0.25 μmole of $Na_4P_2O_7$, 50 nmoles of $MnCl_2$, 0.2 μmole of UDP, and 2.4 nmoles of ^{14}C-sucrose (420 cpm/pmole) in a final volume of 50 μl. The reaction was terminated by spotting on Whatman No. 1 paper strips (2 × 50 cm). The reactions were chromatographed 22 hr in a descending system with 1 M ammonium acetate, pH 3.6, and 95% ethanol (3:7) as solvent. Radioactive sucrose, UDP-glucose, and glucose-1-P were used as standards. The chromatographed strips were divided into 1-inch sections and counted in a liquid scintillation counter. Net radioactivity incorporated into each compound was determined by summing the cpm in the area deter-

Table I. *Properties of Sucrose-P Synthetase*

Chloroplasts were prepared and assayed as described in "Methods." Complete = ^{14}C-fructose-6-P, UDP-glucose, $MgCl_2$, buffer, and chloroplasts in the concentrations indicated in "Methods."

Additions to Reaction	Radioactivity in Sucrose
	cpm
Experiment 1	
Complete	5400
+ alkaline phosphatase treatment	3400
Experiment 2	
Complete	3000
− chloroplasts	600
− UDP-glucose	645
− $MgCl_2$	810
+ invertase	600
Sonicated chloroplasts	650
− ^{14}C-fructose-6-P + ^{14}C-fructose	610
− ^{14}C-fructose-6-P − chloroplasts + ^{14}C-fructose	600

mined by the appropriate standard and subtracting from that value the total cpm in that area on a reaction run in the absence of enzyme.

Protein. This was assayed by the method of Lowry *et al.* (13).

RESULTS AND DISCUSSION

Localization and Properties of Sucrose-P Synthetase in P. aureus. Using the assay described in "Methods," we have examined root, stem, and leaf extracts of *P. aureus* seedling tissues and have been able to detect sucrose-P synthetase activity only in intact chloroplasts. Radioactivity from ^{14}C-fructose-6-P was detected in sucrose when this labeled substrate was incubated in the presence of UDP-glucose with extracts from nonphotosynthetic tissue (roots of light-grown plants or roots and hypocotyls of etiolated seedlings), but this activity was completely abolished by the addition of common phosphatase inhibitors such as fluoride or inorganic phosphate. Thus we conclude that the reaction measured in this case involved the cleavage of fructose-6-P to fructose and subsequent synthesis of sucrose from fructose and UDP-glucose in the reaction catalyzed by sucrose synthetase.

The measurable levels of activity for sucrose-P synthetase in intact chloroplasts were found to be quite low and only partially dependent upon the presence of UDP-glucose in the reaction mixture. It was discovered that washing the chloroplast preparations with buffer containing 10% DMSO enhanced the UDP-glucose-dependent radioactivity detected in sucrose from 1440 cpm in the absence of DMSO wash to 5160 cpm following DMSO wash. Dimethylsulfoxide has been used in the assay of intact plant cells (6) and appears to enhance the permeability of cells to small metabolites. It seems probable that a similar phenomenon is responsible for the DMSO-induced increase in measurable sucrose-P synthetase activity in chloroplasts.

The data of Table I show some of the properties of the sucrose-P synthetase of chloroplasts. No free sucrose-P is demonstrable during the course of the reaction, and all of the product appears as sucrose. Treatment with alkaline phosphatase after the reaction is terminated does not increase the radioactivity in sucrose. Presumably a phosphatase is present in the chloroplasts which rapidly converts sucrose-P to sucrose and P_i. Omission of UDP-glucose or $MgCl_2$ results in almost complete loss of activity. Treatment with invertase has a similar effect, indicating that the reaction product is indeed sucrose. No net production of sucrose is observed when ^{14}C-fructose is substituted for ^{14}C-fructose-6-P, indicating that sucrose synthetase is not present in chloroplasts. However, it should be mentioned that Bird *et al.* (3) have reported the presence of this enzyme activity in the chloroplasts of other plants. We have also shown that when the chloroplasts are broken by sonication, all activity for sucrose-P synthetase is lost. This suggests that the enzyme may be associated in an essential manner with the structural components of the chloroplast. Finally, the data of Table II show that, within the limit of the sensitivity of our assay, the enzyme is quite specific for UDP-glucose; slight activity is obtained with ADP-glucose.

Table II. *Sucrose-P Synthetase Specificity for Nucleoside Diphosphate Sugar*

Chloroplasts were prepared and assayed as described in "Methods."

Nucleoside Diphosphate Glucose Added (2.5 mM)	Radioactivity in Sucrose
	cpm
UDP-glucose	4200
ADP-glucose	900
dTDP-glucose	500
CDP-glucose	630
GDP-glucose	600
None	620

Distribution of Sucrose Synthetase Activity. The finding that sucrose-P synthetase activity is demonstrable only in intact chloroplasts of *P. aureus* where sucrose synthetase is absent is in agreement with the suggestion of others that sucrose-P synthetase and the corresponding phosphatase are the enzymes responsible for the biosynthesis of sucrose from photosynthetically fixed CO_2. The results shown in Table III concerning the distribution of sucrose synthetase activity in *P. aureus* further support this hypothesis. The data show that activity for this enzyme is high only in nonphotosynthetic tissues, that is, in the roots and hypocotyls of etiolated seedlings and in the roots of beans germinated and grown in the light. Activity is significantly lower in photosynthetic tissues, that is, in the hypocotyls, epicotyls, and leaves of light-grown plants. Thus, it seems very unlikely that sucrose synthetase plays a role in the biosynthesis of sucrose from photosynthetically fixed CO_2. Rather, it suggests that this enzyme functions predominantly in catalyzing the conversion of translocated sucrose to nucleoside diphosphate glucoses and fructose in nonphotosynthetic tissues.

Distribution of Pyrophosphorylase Activities in P. aureus. It is known that nucleoside diphosphate glucoses are required not only in nonphotosynthetic tissues but also in photosynthetic tissues. For example, ADP-glucose is accepted to be the precursor of starch and UDP-glucose the precursor of sucrose-P. Since sucrose synthetase levels are very low or barely detectable in photosynthetic tissues, it seems logical to assume that other enzymes must be responsible for catalyzing the synthesis of nucleoside diphosphate glucoses in these tissues. The most obvious candidates for this role are the pyrophosphorylases. This idea is supported by the detailed studies of Ghosh and Preiss (7) on the ADP-glucose pyrophosphorylase, which provide convincing evidence that this enzyme is a major catalyst for the biosynthesis of ADP-glucose in spinach leaves.

We have demonstrated activity in *P. aureus* tissues for the pyrophosphorylases which utilize UTP, ATP, dTTP, GTP, and CTP for the synthesis of the corresponding nucleoside diphosphate glucoses. Table IV shows how the activities for these enzymes are distributed in the various tissues of *P. aureus*. It should be pointed out that, with the exception of the UDP-glucose pyrophosphorylase, we have not performed detailed studies to

Table III. *Distribution of Sucrose Synthetase Activity*
Enzyme was prepared and assayed as described in "Methods."

Tissue	Specific Activity
	nmoles/hr·mg protein
Hypocotyls (etiolated seedlings)	4300
Roots (etiolated seedlings)	3600
Roots (light-grown plants)	3300
Hypocotyls (light-grown plants)	90
Epicotyls (light-grown plants)	41
Leaves (light-grown plants)	Trace

determine the optimum conditions for assay of these enzymes. Therefore, the specific activities presented in Table IV may not represent maximum velocities. However, we have determined that the levels of competing enzymes (phosphodiesterases and phosphatases) are essentially the same in all of the *P. aureus* tissues assayed, and therefore we feel that comparisons of activities of each pyrophosphorylase among different tissues are valid. The data show that, with the dramatic exception of the UDP-glucose pyrophosphorylase, the levels of the pyrophosphorylases are generally lower in nonphotosynthetic tissues than in photosynthetic ones.

The relationships of the pyrophosphorylases and of sucrose synthetase are shown in a different way in Figure 1. In this experiment, beans which were germinated and grown for 4 days in the dark were transferred (at zero time in Fig. 1) to a lighted growth chamber. The change in levels of these enzymes was followed in the hypocotyls. Once again, it can be seen that, although no change is seen in the CDP-glucose pyrophosphorylase, the activity for the ADP-glucose, dTTP, and GDP-glucose pyrophosphorylases increase as the hypocotyl is transformed to a photosynthetic tissue, while the activities for sucrose synthetase and the UDP-glucose pyrophosphorylase decrease with remarkably similar kinetics. This type of result suggests that, in at least some nonphotosynthetic tissues, sucrose synthetase may be a more important catalyst than the pyrophosphorylases in the synthesis of ADP-, dTDP-, and GDP-glucose, and that the converse may be true in photosynthetic tissues. However, this is clearly not a satisfactory explanation for the relationship between sucrose synthetase and the UDP-glucose pyrophosphorylase. In fact, the similar kinetics of change in these two enzymes and the similar pattern of distribution in various tissues suggested to us that these enzymes may function together in the metabolism of nonphotosynthetic tissues.

Sucrose, translocated to nonphotosynthetic cells, may be metabolized by two known pathways. It may be hydrolyzed to glucose and fructose by invertase. But this process results in the loss of the high free energy of hydrolysis of the glycosidic linkage of sucrose. On the other hand, sucrose may be converted by sucrose synthetase to a nucleoside diphosphate glucose and fructose and thus conserve the energy of the sucrose bond in the form of a nucleoside diphosphate glucose. These nucleoside diphosphate glucoses may be used directly for such things as cell wall polysaccharide biosynthesis or the formation of other nucleoside diphosphate sugars.

The uridine-containing nucleoside diphosphate glucose is the probable major product of sucrose synthetase *in vivo* (10). We propose that UDP-glucose is converted by UDP-glucose pyrophosphorylase in the presence of pyrophosphate to glucose-1-P and UTP. The product of this coupled reaction is a phosphorylated form of glucose which may be used in general cell metabolism. In effect, this two-reaction sequence could be used by the plant to transport and accumulate the glucose portion of sucrose.

Table IV. *Distribution of Pyrophosphorylase Activities*
Enzymes were prepared and assayed as described in "Methods."

Tissue Used as Source of Enzyme	Specific Activity				
	UDP-glucose	ADP-glucose	dTDP-glucose	CDP-glucose	GDP-glucose
	nmoles/hr·mg protein				
Roots and hypocotyls (etiolated)	68,000	1.5	5.3	1.0	4.5
Roots (light-grown plants)	24,600	2.3	16.1	0.2	6.9
Hypocotyls (light-grown plants)	7,300	7.3	14.6	1.2	24.3
Epicotyls (light-grown plants)	735	14.7	14.5	6.8	36.1

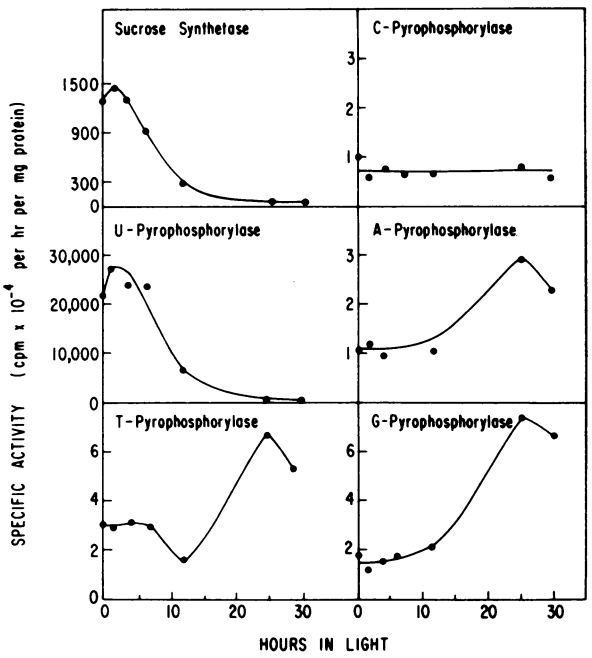

FIG. 1. The change in activity of sucrose synthetase and of the pyrophosphorylases in hypocotyls of etiolated seedlings following exposure of dark-grown plants to light. Four-day-old etiolated *P. aureus* seedlings were placed in a lighted growth chamber at zero time. Five grams of hypocotyls were sampled at the times indicated, and the enzymes were prepared and assayed as described in "Methods." Protein concentrations in the preparations did not vary significantly during the course of the experiment.

Table V. *Coupled Reactions with Sucrose Synthetase and UDP-glucose pyrophosphorylase*

Partially purified preparations of sucrose synthetase and UDP-glucose pyrophosphorylase were prepared as described in "Methods." Complete = ^{14}C-sucrose, UDP, $MnCl_2$, $Na_4P_2O_7$, buffer, and enzymes present in the concentrations indicated in "Methods."

Reaction Mixture	UDP-glucose Formed	Glucose-1-P Formed
	cpm	
Complete	3,600	28,000
− UDP	3,400	2,500
− $Na_4P_2O_7$	53,900	2,400
− pyrophosphorylase	48,100	2,000

A similar suggestion has been made by Gibbs (8) in his review of carbohydrate metabolism in plants.

An advantage of this conversion is that the steady state level of UDP-glucose will be kept at a minimum by the excessively high levels of UDP-glucose pyrophosphorylase. This fact is important since UDP-glucose is a potent inhibitor of the other reactions of sucrose synthetase, that is, the synthesis of ADP-, dTDP-, CDP-, and GDP-glucose (10). Therefore, UDP-glucose pyrophosphorylase, by keeping the levels of UDP-glucose low, increases the sucrose synthetase-catalyzed synthesis of ADP-, dTDP-, CDP-, and GDP-glucose.

One would logically expect these reactions to occur at the cell membrane. Support for this proposal comes from the fact that both sucrose synthetase and UDP-glucose pyrophosphorylase possess physical properties which suggest that they may be membrane-associated. Sucrose synthetase in *P. aureus* exists as a particle of roughly 1 million molecular weight which possesses a density of 1.21. This suggests that the sucrose synthetase particle contains as much as 36% lipid-like material (10). The UDP-glucose pyrophosphorylase is also unusually large, with a molecular weight of about a half-million; preliminary determinations of the density of this enzyme by equilibrium sedimentation in a 5 to 70% sucrose gradient yielded an approximate value of 1.12, suggesting an extremely high content of lipid-like material.

One point critical to the development of this hypothesis would be the availability in the cells of pyrophosphate as substrate for the pyrophosphorylase reaction. A measurable amount of pyrophosphatase is present in these cells, and, although not examined by us yet, it may be likely that insufficient levels of free pyrophosphate exist to support this reaction. However, because of the possibility of these reactions occurring in the cell membrane, we feel it is reasonable to suggest the existence of a pyrophosphate-generating system localized adjacent to, or perhaps even within, one of these particles. In fact, the entire reaction sequence could be considered cyclic by using the UTP generated in the reaction as a source of pyrophosphate and by using the UMP thus generated in conjunction with another UTP to produce two UDPs as substrate for sucrose synthetase.

It is interesting that the conversion of sucrose to glucose-1-P by sucrose synthetase and UDP-glucose pyrophosphorylase has recently been proposed by DeFekete (5) as a probable pathway for the conversion of sucrose to starch in maturing cotyledons of *Vicia faba*. DeFekete also discussed the problem of the availability of pyrophosphate to support the pyrophosphorylase reaction, and she suggested that sufficient levels may exist under certain conditions.

We have just begun to study the conversion of sucrose to glucose-1-P using a combination of a 100-fold purified preparation of sucrose synthetase and a 256-fold purified preparation of UDP-glucose pyrophosphorylase. These preliminary results are shown in Table V. ^{14}C-Sucrose, unlabeled UDP, sodium pyrophosphate, and $MnCl_2$ were incubated with a mixture of limiting sucrose synthetase and excess UDP-glucose pyrophosphorylase in the approximate ratio of activity found in cell extracts. The products of the coupled reaction were separated by descending paper chromatography. When both enzymes and all of these substrates were present in the reaction, glucose-1-P is formed as the primary reaction product. If UDP is omitted, the first reaction, that catalyzed by sucrose synthetase, cannot proceed, and neither product is formed. If pyrophosphate is omitted, the primary product formed is, as expected, UDP-glucose. If the UDP-glucose pyrophosphorylase is omitted from the reaction, one obtains essentially the same result as when pyrophosphate is omitted.

Admittedly, the above data do not constitute proof of our hypotheses. Although detailed kinetic studies of sucrose synthetase in *P. aureus* have been reported for the reaction in the direction of sucrose synthesis (10), such work has not yet been accomplished for the reverse reaction. One of us (D. D.) has recently purified this enzyme to electrophoretic homogeneity, and kinetic studies of the reverse reaction, the synthesis of the nucleoside diphosphate glucoses, are in progress. At present, it appears that the kinetic characteristics of the reaction in this direction are very complex. However, we can state that, at equimolar concentrations of different nucleoside diphosphates, the relative velocities of the reactions are similar to those observed for the synthesis of sucrose, namely UDP > ADP $\cong$ TDP > CDP > GDP. In addition to a more detailed examination of the kinetics of the sucrose synthetase-catalyzed synthesis of nucleoside diphosphate glucoses, some type of proof of the availability of pyrophosphate to support the proposed coupled sequence would be desirable, as would the results of experiments *in vivo* designed to study the immediate fate of labeled sucrose supplied to nonphotosynthetic tissues.

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