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SUCROSE AND HEXOSE METABOLISM BY CLUBS OF *TYPHONIUM GIRALDII* AND ROOTS OF *PISUM SATIVUM*

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Key Word Index—*Typhonium giraldii*; Araceae; spadix; *Pisum sativum*; Leguminosae; pea; roots; sucrose; glucose; fructose; metabolism.

Abstract—The maximum catalytic activities of acid invertase, alkaline invertase and sucrose synthase were shown to exceed estimates of the rate of sucrose breakdown in clubs of the spadix of *Arum maculatum*. [Glucosyl-^{14}C]sucrose, [fructosyl-^{14}C]sucrose, [^{14}C]glucose, and [^{14}C]fructose were supplied to developing clubs of the spadix of *Typhonium giraldii* and the apical 36 mm of roots of seedlings of *Pisum sativum*. The detailed distribution of ^{14}C revealed no differences between [glucosyl-^{14}C]sucrose and [fructosyl-^{14}C]sucrose, or between [^{14}C]glucose and [^{14}C]fructose. It is argued that the glucosyl group of sucrose is used for starch and structural polysaccharide synthesis and for respiration as readily as is the fructosyl group; and that the same lack of discrimination is found between free glucose and free fructose.

INTRODUCTION

Most of the starch made by higher plants is synthesized from translocated sucrose in non-photosynthetic cells [1]. Initial metabolism of this sucrose occurs via sucrose synthase, alkaline invertase and acid invertase, acting singly or in combination. We do not know whether the products of sucrose synthase, UDPglucose and fructose, are equally available for starch synthesis. We also lack this information for the invertases as we have little information about whether glucose and fructose are always metabolized in the same way. It may not be assumed that the two halves of the sucrose molecule will behave identically. The metabolism of UDPglucose could differ from that of fructose. Similarly, as plants contain a range of hexose kinases [2], the metabolism of fructose could differ from that of glucose. A specific pathway for fructose metabolism operates in some animal tissues [3].

The aim of the work in this paper was to investigate the relative contributions of the two halves of the sucrose molecule to starch synthesis and to other pathways in two plant tissues. The latter were the developing club of the spadix of thermogenic Araceae, where starch synthesis dominates metabolism; and pea roots, where starch synthesis is merely one of a number of quantitatively significant fates of sucrose. We used two closely related species of the Araceae, *Arum maculatum* and *Typhonium giraldii*, and regard their metabolism as being equivalent [4]. Our experimental approach was to estimate the maximum catalytic activities of the three enzymes that can metabolize sucrose, and to determine the detailed distribution of label after supplying the tissues with [glucosyl-^{14}C]sucrose, [fructosyl-^{14}C]sucrose, [^{14}C]glucose and [^{14}C]fructose. The latter type of information is available for pea epicotyls but this is a tissue in which there is no significant labelling of starch [5].

RESULTS AND DISCUSSION

Enzyme activities

Extracts of β-stage clubs of *Arum maculatum* were assayed for invertase at intervals of 0.5 pH unit from pH 4.0 to 8.0. Peaks of activity were found at pH 5.0 and 7.5 and are attributed to acid and alkaline invertase, respectively. In a recombination experiment we measured both invertases in an extract of β-stage and pre-thermogenic *Arum* clubs. The values obtained were within 15% of those predicted from measurements made on separate samples of the two types of club. This suggests that homogenization and extraction did not lead to significant losses of invertase activity. Sucrose synthase was assayed with UDP as this is its likely substrate *in vivo* [6]. For sucrose synthase we optimized the pH of the assay mixture, and the concentration of each of its components with extracts of β-stage clubs. Recombination experiments, as per invertase, gave values for the mixture that were within 25% of those predicted.

Measurements of enzyme activity and estimates of the rate of sucrose breakdown *in vivo* are compared in Table 1. Previous work [7] and data presented in the next section suggest that there are only two quantitatively significant fates for sucrose in the developing club, conversion to starch and entry into the respiratory pathways. We have measured respiration [7] and starch accumulation [8] in *Arum* clubs. Our results in Table 2 suggest that up to two-thirds of the substrate that enters the respiratory pathways is withdrawn from those pathways for biosynthesis. Thus to calculate sucrose

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Table 1. Comparison of activities of enzymes of sucrose metabolism in extracts of β-stage clubs of *Arum maculatum* with an estimate of the rate of sucrose breakdown *in vivo*

	Rate (μ mol sucrose consumed/min per club at 12°)
Acid invertase *	0.58 ± 0.12 (5)
Alkaline invertase *	0.65 ± 0.11 (5)
Sucrose synthase *	0.26 ± 0.05 (4)
Starch synthesis†	0.047
Respiration‡	0.032
Sucrose consumption	0.079 (0.047 + 0.032)

* Values are means ± s.e. from the number of clubs shown in parentheses.

† Data from ref. [8].

‡ Data from ref. [7].

consumption by respiration we have assumed that each molecule of sucrose that entered the respiratory pathways produced four molecules of carbon dioxide. All estimates are reduced to those expected at 12°, the temperature at which starch accumulation was measured and the natural temperature of growth of the club [8]. A Q_{10} of two was assumed. It can be seen that the sum of sucrose consumption for starch synthesis and respiration is appreciably less than the maximum catalytic activity of each enzyme. Thus any one of the three enzymes could be responsible for the initial breakdown of sucrose. Such detailed information is not available for pea roots, but we have shown that the maximum catalytic activities of sucrose synthase and alkaline invertase are comparable [9] and exceed the rate of respiration [10].

Arum is a marked contrast to two other tissues where starch synthesis is the major fate of sucrose. In developing tubers of potato [11] and embryos of pea [12], sucrose synthase is by far the dominant enzyme, and in potato at least invertase activity appears too low to make a major contribution to sucrose metabolism. The cytosolic location of sucrose synthase and alkaline invertase [6] suggests that these are the enzymes most likely to determine the initial fate of translocated sucrose. High sucrose synthase activity correlates with extensive metabolism of translocated sucrose, regardless of whether starch is the main product. This suggests a key role for this enzyme in sucrose partitioning in general. A mechanism for such a role has been proposed [11, 12]. However the role of alkaline invertase is not apparent as there is no clearly established relationship between its activity and the fate of sucrose.

Metabolism of labelled sugars

Clubs were labelled under conditions as close to natural as was practicable. To some degree excised clubs can be cultured *in vitro* merely by placing their short stalk in 0.3–0.5 ml culture medium. Ten clubs of *Arum maculatum*, initial fresh weight 105–692 mg, were incubated in this way for 10 days: the increase in fresh weight was 31 ± 9% (mean ± s.e.). When *Arum* clubs were incubated for 3.5 hr in [^{14}C]sucrose or in [^{14}C]glucose, the ^{14}C per mg fresh weight recovered in the distal half of the club was similar to that in the proximal half. We suggest that our procedure led to the translocation of the supplied sugar to the whole of the club.

The distribution of ^{14}C when labelled sugars were supplied to the developing clubs of *Typhonium giraldii* is shown in Table 2. Label is only attributed to starch when it had been shown to be present as [^{14}C]glucose released by treatment of the ethanol-insoluble material with amyloglucosidase and α-amylase. To compare different clubs, the label in each fraction of a club is expressed as a percentage of the total ^{14}C metabolized by that club. The latter is the sum of the ^{14}C recovered as $^{14}CO_2$, and in the ethanol-insoluble fraction; plus the acidic, basic and neutral components of the ethanol-soluble fraction minus that still present as the labelled sugar fed to the club. The total of the ^{14}C metabolized plus that remaining in the fed sugar was calculated for each club and expressed as a percentage of the ^{14}C supplied at the start

Table 2. Distribution of ^{14}C after supplying [U-^{14}C-glucosyl]sucrose, [U-^{14}C-fructosyl]sucrose, [U-^{14}C]glucose, and [U-^{14}C]fructose to clubs of *Typhonium giraldii* in culture *in vitro*

	^{14}C per fraction as % of total ^{14}C metabolized							
Fraction	[Glycosyl-^{14}C]sucrose		[Fructosyl-^{14}C]sucrose		[^{14}C]Glucose		[^{14}C]Fructose	
CO_2	9	5	11	5	1	6	5	8
Ethanol-insoluble material	75	85	71	73	57	60	49	50
Starch	65	77	62	69	49	44	44	43
Protein			9		4			5
Ethanol-soluble material								
Basic components	6	4	8	4	7	11	5	6
Acidic components	4	4	5	4	6	6	4	5
Sucrose					21	13	28	20
Glucose	0.3	0.2	0.1	0.3			2	2
Fructose	0.1	0.1	0.3	1.1	3	3		
^{14}C Metabolized:								
as dpm × 10^{-6}	2.06	1.73	1.37	1.83	1.53	2.31	1.39	1.5
as % ^{14}C supplied	58	49	42	56	54	82	61	70
Fresh weight of club (g)	1.45	0.63	1.29	0.83	0.76	2.1	0.8	1.5

Incubation was for 22 hr.

of the experiment. A value of 100.9 ± 1.8 (mean ± s.e.m. for 13 clubs) was obtained; thus the data in Table 2 are not significantly affected by losses during extraction and analysis. As appreciable amounts of the labelled sugars remained at the end of the experiments, it is also unlikely that our labelling patterns reflect limited availability of substrate.

Clubs were analysed after 14 and 22 hr labelling. At 14 hr the amount of label metabolized was less than at 22 hr but its distribution, when expressed as percentage metabolized, was almost identical at 14 and 22 hr. Thus data are presented only for 22 hr. When [^{14}C]sucrose was supplied almost two-thirds of that metabolized appeared in starch, which together with protein accounted for almost all the label in the insoluble fraction. Thus very little of the sucrose was converted to structural polysaccharides in the clubs. The remaining metabolized [^{14}C]sucrose was divided between CO_2, organic acids and amino acids in the manner expected of a growing plant tissue. The crucial aspect of the data for labelled sucrose is that there is no indication that starch, or any other component of the club, was labelled preferentially by either of the hexosyl moieties of sucrose. The labelling pattern obtained with [^{14}C] hexoses was comparable to that found with [^{14}C]sucrose, except that the hexoses made a smaller contribution to the labelling of starch and a substantial contribution to the labelling of sucrose. Again the central point is that no significant differences could be detected between [^{14}C]glucose and [^{14}C]fructose.

With peas we removed the cortex from the region of the root 36–46 mm from the apex, supplied the sugar directly to the bared stele, and analysed the distribution of label in the apical 36 mm of the root. Previously we showed that sucrose is absorbed and translocated by the stele, and then transported from the stele to the apical and cortical cells in the root tip [9,13]. This transport occurs in the symplasm and the whole process occurs without detectable hydrolysis of the sucrose. We suggest that our procedure labels the root apex via its normal transport system.

The labelling patterns for pea roots after a five-hr incubation is shown in Table 3. ^{14}C per fraction is again expressed as a percentage of that metabolized. At least 90% of the label in the insoluble, and the soluble, fractions was recovered in their components obtained by further analysis. Thus the labelling patterns are not seriously affected by losses during analysis. The general distribution of label from both [^{14}C]sucrose and [^{14}C] hexose is that expected of a growing tissue in which cell wall synthesis is appreciable, and is comparable to that reported earlier [9]. We stress three particular features of the patterns. First, the two halves of the sucrose molecule made comparable contributions, not only to starch, but also to the different structural polysaccharides and to all the remaining fractions analysed. Second, no differences could be demonstrated between the patterns produced by [^{14}C]glucose and [^{14}C]fructose. Third, [^{14}C]sucrose was a better precursor of cellulose than of starch, whilst the reverse was true of the labelled hexoses as each labelled starch more than cellulose.

Our results from two very different tissues agree very closely and support the following conclusions. First, we found no evidence that fructose supported metabolism in any way that was different from that found for glucose. The two hexoses appeared to be equivalent regardless of the possibility of differences in the activities, affinities and intra-cellular distribution of glucokinase and fructokinase. It seems likely that in these tissues, at least, the activities of the kinases are sufficient to ensure rapid conversion of glucose and fructose to their respective 6-phosphates, which are then rapidly equilibrated by glucose-6-phosphate isomerase. Evidence for the latter is provided by comparing mass action ratios and equilibrium constants [14].

Our second conclusion is that the fructosyl and glucosyl groups of sucrose are equally available not only for starch synthesis but also for the synthesis of structural

Table 3. Distribution of ^{14}C after supplying [U-^{14}C-glucosyl]sucrose, [U-^{14}C-fructosyl]sucrose, [U-^{14}C]glucose, and [U-^{14}C]-fructose to the apical 36 mm of roots still attached to plants of *Pisum sativum*

Fraction	^{14}C per fraction as % of total ^{14}C metabolized			
	[Glucosyl-^{14}C]sucrose	[Fructosyl-^{14}C]sucrose	[^{14}C]Glucose	[^{14}C]Fructose
CO_2	24	13	12	8
Ethanol-insoluble material	36	42	40	48
Starch	9	10	21	20
Protein	3	3	4	5
Cellulose	16	20	3	3
Hemicellulose	8	7	4	7
Pectin	3	3	7	8
Ethanol-soluble material				
Basic components	12	11	5	5
Acidic components	10	10	17	7
Neutral components – sucrose	17	24		
Neutral components – glucose			26	
Neutral components – fructose				32
^{14}C Metabolized:	534	371	479	2598
as dpm $\times 10^{-3}$				
as %^{14}C supplied	5.2	3.3	9.3	9.9
Fresh weight of sample (mg)	874	905	858	805

polysaccharides and for respiration. We found no significant differences in the labelling patterns obtained with the differently labelled sucrose molecules. The similar metabolism of both groups of the sucrose molecule might be construed as evidence that the initial cleavage of sucrose was via invertase not sucrose synthase. This temptation should be resisted for two reasons. First, we have presented evidence that both the tissues we studied contained sufficient sucrose synthase to mediate sucrose breakdown. Second, the labelling patterns we found are completely compatible with initial breakdown by sucrose synthase. The glucosyl group of UDPglucose could be converted very rapidly to glucose 6-phosphate via UDP-glucose pyrophosphorylase and phosphoglucomutase. Evidence for the operation of such a pathway in two starch storing tissues, pea embryos [15] and potato tubers [11], has already been presented. Some evidence for a major role for sucrose synthase in sucrose breakdown in *Typhonium* clubs is provided by our evidence that sucrose is a better precursor of polysaccharide than is either glucose or fructose (Table 2).

EXPERIMENTAL

Materials. [U-^{14}C glucosyl]sucrose and [U-^{14}C fructosyl]sucrose were from New England Nuclear, Dupont (U.K.) Ltd., Southampton; [U-^{14}C]glucose and [U-^{14}C]fructose were from Radiochemical Centre, Amersham. Influorescences of *Arum maculatum* L. and *Typhonium giraldii* Engler were from plants growing in local natural sites and the University Botanic Garden, Cambridge, respectively. Only the clubs of the spadices were analysed, and these were excised within 2 hr of collecting the spadices and were used within 5 min of excision. The developmental stages were defined and recognized as in ref. [7]. Peas, *Pisum Sativum* L. cv Kelveldon Wonder, were grown as in ref. [16] and used at day 5.

Enzyme assays. Tissue was homogenized, first with a pestle and mortar, and then with a ground-glass homogenizer. Examination of the homogenates with a microscope revealed very few unbroken cells. For invertase 0.7–0.8 g fr. wt was homogenized in 3 vol. 0.19 M Na_2HPO_4–2.75 mM citric acid, pH 8.0: the homogenate was centrifuged at 35 000 *g* for 30 min, and the supernatant dialysed for 24 hr against 5 l 18 mM Na_2HPO_4–0.8 mM citric acid, pH 7.5 and then assayed as in ref. [17] at 30° in a reaction mixture that contained 150 μl extract, 3.75 mg sucrose and 150 μl of either 0.104 M Na_2HPO_4–48 mM citric acid, pH 5.0 or 0.18 M Na_2HPO_4–8 mM citric acid, pH 7.5. Extraction and dialysis were at 1–4°. For sucrose synthase 0.2–0.9 g fr. wt of tissue was homogenized in 3 vol. 100 mM Tris–HCl, pH 7.6, 20 mM EDTA, 20 mM cysteine–HCl, 20 mM diethyldithiocarbamate. The homogenate was centrifuged at 35 000 *g* for 15 min and the supernatant desalted by passage through a column (1 × 16 cm) of Sephadex G-25 (coarse) equilibrated with 10 mM Tris–HCl, pH 7.0, and then assayed as in ref. [18] in a reaction mixture, 100 μl, that contained 33.3 mM Tris–HCl, pH 8.5, 13.3 mM UDPglucose, 25 mM sucrose, 10 mM [U-^{14}C]fructose (8 Ci/mol). Assay was for 30 min at 25°; extracts were prepared at 1–4°.

Metabolism of ^{14}C sugars. Clubs of Araceae were excised just above the ring of sterile hairs and each planted singly with its stalk in 320 μl medium contained in a centre well (vol. 800 μl) of a 100 ml Erlenmeyer flask. The main compartment of the latter contained 2 ml H_2O and a vial (vol. 1 ml) that contained 0.5 ml 10% (w/v) KOH to absorb $^{14}CO_2$. The Erlenmeyer flask was closed with a cotton wool plug and incubated in the dark at 20°. At the end of each incubation a portion of the culture medium was plated onto medium of identical composition, except that it contained agar at 1.2% (w/v), and incubated at 25° for 3 weeks. Data are presented only for samples in which no infection was detected. The culture medium contained a sugar and 67 mM asparagine, 24 mM homoserine, 29 mM serine, 23 mM glutamine, 21 mM alanine, 100 mM glycine plus the inorganic components, growth factors and micronutrients from the medium in ref. [19]. The sugar components were: *Arum*, 0.53 M sucrose; *Typhonium*, 0.53 M [glucosyl-U-^{14}C]sucrose (9.36 mCi/mol), 0.53 M [fructosyl-U-^{14}C]sucrose (8.03 mCi/mol), 0.53 M sucrose plus 0.48 mM [U-^{14}C]glucose (9.35 Ci/mol), 0.53 M sucrose plus 0.48 mM [U-^{14}C]fructose (7.08 Ci/mol).

For experiments with peas we took replicate samples of 20 5-day-old seedlings, removed the cortex from the region of the roots 36–46 mm from the apex and put the sample in a Perspex box (20 × 10 × 3 cm) that was divided lengthwise into three compartments. There were holes in the divisions between compartments and the seedlings were arranged with their roots passing through each compartment. The first compartment contained the apical 36 mm of the roots, the second the region where the stele was bared, and the third the rest of the seedling supported in damp cotton wool. The roots were sealed into their compartments and a strip of Whatman's No.3 chromatography paper was laid across the bared steles as in ref. [13]. Incubations were started by adding to the paper 0.5 ml 0.02 M KH_2PO_4, pH 5.2, that contained: 1.5 mM [glucosyl-U-^{14}C]sucrose (6.4 Ci/mol), 1.5 mM [fructosyl-U-^{14}C]sucrose (6.38 Ci/mol), 0.3 mM [U-^{14}C]fructose (33.3 Ci/mol). The boxes were closed and sealed, and a stream of air was passed over the roots and then through 25 ml 10% (w/v) KOH to collect $^{14}CO_2$. The samples were incubated in the dark at 25° for 5 hr.

At the end of the above incubations the apical 36 mm of the roots were excised, and both roots and clubs were killed with boiling 80% (v/v) aq. EtOH. All samples were extracted, 2 × before and 3 × after homogenization, with 20 ml portions of boiling 80% (v/v) aq. EtOH. The extracts were combined and evaporated *in vacuo* at 28° to give a suspension that was centrifuged at 32 000 *g* for 30 min. The sediment obtained was added to the material insoluble in 80% EtOH to give the EtOH-insoluble fraction. The supernatant is the EtOH-soluble fraction and was fractionated by ion-exchange and paper chromatography as in ref. [20]. ^{14}C in starch was measured as in ref. [21] and in protein as in ref. [12]. Cell wall components of the insoluble material from peas were obtained by a modification of the method in ref. [22]. Insoluble material, equivalent to 200 mg fr. wt, was refluxed in 20 ml 2% (w/v) sodium hexametaphosphate, pH 3.8, for 4 hr. This suspension was then centrifuged at 20 000 *g* for 20 min. The supernatant contained the pectin: the sediment was dried at 60° and 40–50 mg were extracted under N_2 with 25 ml 24% (w/v) KOH at 25° for 4 hr. The latter extract was then centrifuged at 20 000 *g* for 20 min. The sediment was the cellulose fraction; the supernatant contained the hemi-cellulose. The pectin and the hemi-cellulose fraction were, separately, made up to 100 ml, and 30 ml portions of each were treated as follows to remove starch. The pH was adjusted to pH 7.0 and 5000 units of pig pancreas α-amylase, 0.25 g NaCl and a drop of toluene were added. After incubation at 25° overnight the fractions were dialysed against 6 × 2 l de-ionized (so as to avoid inderactions with Ca^{2+}) H_2O for 24 hr at 4°, reduced to 2–5 ml *in vacuo* at 28° and finally dried at 60° and then counted. ^{14}C in insoluble fractions was solubilized, and all ^{14}C was measured as in ref. [17].

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