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SUCROSE METABOLISM BY ROOTS OF *PISUM SATIVUM*

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Abstract—The aim of this work was to relate [^{14}C]-sucrose metabolism to the activities of sucrose synthetase and acid and alkaline invertases in roots of *Pisum sativum*. We fed [U-^{14}C]-sucrose to 5-day-old plants and then excised the apical 6 mm of the roots and dissected the regions 6–24 mm from the root apices into stele and cortex. The detailed distribution of ^{14}C in these parts of the roots was determined at the end of the feeding period and after a chase. The data indicate that sucrose arriving in the stele is divided between storage, conversion to polysaccharide, and consumption in respiration, whereas sucrose arriving in the rest of the root is used in respiration or converted to polysaccharide or hexose so rapidly that little is stored. Fractionation of carefully prepared extracts of pea roots, tubers of *Solanum tuberosum*, and spadices of *Arum maculatum* showed that sucrose synthetase was recovered in the soluble fraction. The results are discussed in relation to the roles of the aforementioned enzymes.

INTRODUCTION

Sucrose is the major carbohydrate translocated to pea roots [1]. This sucrose moves from the stele to the cortex and apex of the root as sucrose via the symplasm [2]. Thus all the cells of the pea root depend directly upon sucrose as their main source of carbon for polysaccharide synthesis and respiration, and it is the breakdown of sucrose, rather than the metabolism of hexose, which must be regarded as the starting point of carbohydrate metabolism in the pea root. These roots contain at least three enzymes capable of breaking down sucrose, sucrose synthetase [E.C.2.4.1.13], acid invertase and alkaline invertase [3,4]. Specific roles have been proposed for these enzymes [5]. It was suggested that sucrose synthetase is concerned mainly with the conversion of sucrose to polysaccharide via sugar nucleotides, whilst the invertases are principally involved in supplying hexose for respiration. Hydrolysis of sucrose in cells with a marked demand for hexose was attributed to acid invertase located in the storage compartment, and high acid invertase activity was seen as a means of diverting sucrose to hexose and preventing substantial accumulation of sucrose. It was also suggested that cells with low acid invertase activity obtain hexose for respiration via an alkaline or neutral invertase in the cytoplasm. Previous studies showed that the apical 24 mm of pea roots may be dissected into apex, cortex, and stele, and that these parts differ appreciably in sugar content and in the activities of the above enzymes [3,4]. The aims of the work reported in the present paper were to determine the labelling pattern after supplying [^{14}C]-sucrose to the different parts of the pea root and to compare these patterns with sugar content and the activities of sucrose synthetase and acid and alkaline invertase. Our results indicate how the different parts of the pea root deal with their supply of sucrose and whether this is in agreement with the roles proposed for the above enzymes.

Most of our results are from pulse and chase experiments designed to follow the fate of [^{14}C]-sucrose in the different parts of the root. We labelled the root by removing the cortex 36–46 mm from the apex and supplying [^{14}C]-sucrose directly to the exposed stele of an otherwise intact seedling. We have shown that such [^{14}C]-sucrose is absorbed and translocated by the stele to the apical 24 mm of the root and that it moves from the stele via the symplasm into the surrounding cortical and apical cells. We have also shown that this uptake and transport occur without cleavage of the [^{14}C]-sucrose [2]. This technique ensures that the cells in the apical 24 mm of the root are supplied with [^{14}C]-sucrose in the same way as they receive sucrose in the intact plant. At the end of this pulse, the apical 6 mm of the roots were excised, and the regions 6–24 mm from the apices were separated into stele and cortex. The distribution of ^{14}C in the different regions of the root was determined at once or after a chase during which the different parts of the root were incubated, separately, in 0·02 M KH_2PO_4.

RESULTS AND DISCUSSION

Table 1 combines data from previous experiments and the present work and shows the distribution of the enzymes of sucrose breakdown and the general properties of the apical 24 mm of the pea root. Both the apical 6 mm and the cortex have appreciable activities of sucrose synthetase and alkaline invertase which are dwarfed by very high activities of acid invertase. If our hypothesis is correct, then some of the sucrose arriving in these tissues would be used at once for polysaccharide synthesis and the rest would be rapidly hydrolysed to hexose which would be used in respiration or stored. In the steles the activities of sucrose synthetase and alkaline invertase are higher than in the rest of the root whilst that of acid invertase is low, both in relation to the other

Table 1. Properties of different regions of the apical 24 mm of the roots of 5-day-old peas

Property	Stele 6–24 mm from apex	Cortex 6–24 mm from apex	Apical 6 mm
*Sugar content (mg/g fr. wt)			
Sucrose	10·9	1·8	2·9
Glucose	4·7	10·6	5·1
Fructose	0·7	1·2	1·1
†Enzyme activity (nkat/mg protein)			
‡Sucrose synthetase	0·57 ± 0·03	0·28 ± 0·05	0·31 ± 0·01
Acid invertase	0·18	1·51	1·66
Alkaline invertase	0·44	0·32	0·33
CO_2 production (μl/g fr. wt/hr)	500	251	565

* Data from ref. [3]. † Data on invertase from ref. [3]. ‡ Values are means ± S.E. of assays of at least 3 extracts.

two enzymes in the stele and to the activity of acid invertase in the rest of the root. We predict that sucrose arriving in this region of the stele would be used for polysaccharide synthesis and for respiration but neither use would preclude appreciable storage.

The above predictions were tested in a series of pulse and chase experiments. We supplied [U-^{14}C]-sucrose to samples of 20 plants and then took 10 of the plants for the pulse and the other 10 for the chase. The replication between samples was adequate and can be assessed by comparing pulse and chase within an experiment, and pulses of the same duration in different experiments (Tables 2–4). The amount of ^{14}C recovered from comparable samples sometimes varied but the distribution of ^{14}C in comparable samples showed little variation. The labelling patterns shown in Tables 2–4 were obtained consistently with differently grown batches of seedlings.

Heavy and persistent labelling of sucrose is the most striking feature of the results obtained for the stele (Table 2). Even after pulses as long as 3 hr nearly half of the ^{14}C in the stele was present as [^{14}C]-sucrose. During the chases label moved out of [^{14}C]-sucrose slowly so that after a 3 hr chase [^{14}C]-sucrose still accounted for 33–45% of the label in the stele. Polysaccharides became increasingly labelled with time and a high percentage of the label that did move out of [^{14}C]-sucrose was recovered in polysaccharides. An estimate of the label which moved from [^{14}C]-sucrose into the respiratory pathways can be obtained by summing the ^{14}C recovered as $^{14}CO_2$, in the acidic and basic components of the water-soluble substances, and that present in the insoluble substances which is not accounted for as polysaccharide. Except in the 6 hr chase this estimate is barely more than 25% of the total ^{14}C recovered from the stele. Apart from the 6 hr chase our results show that the [^{14}C]-sucrose which arrived in the stele of the apical 24 mm of the root was not readily metabolized. The metabolism of sucrose which did occur contributed more or less equally to polysaccharide synthesis and to respiration.

We think that the more extensive metabolism of the [^{14}C]-sucrose which occurred in the 6 hr chase may be attributed to depletion of the sugar content of the steles caused by the fact that they were severed from their normal supply of sucrose during the chase. CO_2 production

Table 2. Distribution of ^{14}C in steles of pea roots supplied with [U-^{14}C]-sucrose

	Distribution of ^{14}C									
Experiment no.*	1		2		3		4		5	
Incubation time (hr)	Pulse 1	Chase 2	Pulse 1	Chase 3	Pulse 2	Chase 2	Pulse 2	Chase 3	Pulse 3	Chase 6
^{14}C recovered from steles (dpm × 10^{-3})	6·85	7·45	10·73	6·17	26·07	30·10	22·02	32·15	47·95	40·82
% recovered ^{14}C found in:										
CO_2	—	1·8	—	2·5	—	2·9	—	4·5	—	9·4
Water-soluble substances	96·6	87·2	95·4	83·9	87·4	71·7	83·9	65·0	78·3	47·1
Acidic and basic components	7·9	10·7	7·9	18·1	9·7	17·8	13·1	18·8	12·7	20·6
Neutral components	87·5	76·5	87·5	65·9	77·7	53·9	70·8	46·2	65·6	26·5
Sucrose	71·3	63·1	72·3	45·3	65·4	38·3	60·9	33·3	44·2	10·5
Glucose	6·0	5·1	5·9	7·3	5·3	7·7	4·1	5·2	12·2	6·2
Fructose	5·7	5·0	4·6	9·0	2·3	5·2	3·3	5·3	6·1	6·2
Water-insoluble substances	3·4	11·0	4·6	13·6	12·6	25·4	16·1	30·5	21·7	43·5
Polysaccharides	—	—	—	—	11·1	17·1	11·9	28·8	13·6	25·8

* Stele, cortex, and apex from similarly numbered experiments were derived from the same roots.

by the isolated steles is equivalent to the complete oxidation of 0·61 mg hexose/g fr. wt/hr (Table 1). The labelling of CO_2 and the different cellular components shown in Table 2 indicates that at least a third of the hexose which entered the respiratory pathways was used for biosynthesis. Consequently only two thirds of the hexose carbon would be released as CO_2. Thus the values for CO_2 production in Table 1 indicate that hexose was consumed in respiration at rates approaching 1·0 mg hexose/g fr. wt. per hr. This would exhaust the free hexose in the stele in 6 hr and almost certainly lead to consumption of stored sucrose (Table 1) and thus account for the low labelling of [^{14}C]-sucrose in the 6 hr chase.

The results for the cortex (Table 3) and the apical 6 mm of the root (Table 4) are sufficiently similar to be discussed together. The salient feature of these results is the evidence that the [^{14}C]-sucrose which arrived in the cortex and apex was very rapidly metabolized. [^{14}C]-sucrose accounted for a relatively small percentage of the label recovered from these tissues at the end of the pulses. The values were much lower than those found in the steles. The [^{14}C]-sucrose which was present at the end of the pulses was very rapidly metabolized in the subsequent chases. A chase of 3 hr sufficed to reduce the label recovered as [^{14}C]-sucrose to 5% of the total or less. The decline in the labelling of [^{14}C]-sucrose was faster in the more rapidly respiring apical 6 mm than in the cortex. If we make the same assumptions about the relationship between CO_2 production and the consumption of hexose in respiration as we did for steles, then we can calculate that the apical 6 mm and the cortex respired hexose at rates of 1·04 and 0·46 mg hexose/g fr. wt/hr, respectively, during the chase. Comparison of these values with the sugar contents of the tissues (Table 1) shows that it is unlikely that the rapid fall in the labelling of sucrose in the chases was due to exhaustion of the available supply of sugars. The fate of the [^{14}C]-sucrose metabolized by the cortex and apex may be inferred from Tables 3 and 4. In both cortex and apex the labelling of the polysaccharides was comparable to that found in the steles. The remainder of the label, in both tissues, was recovered as hexose or as compounds derived from the respiration of hexose. The percentage of the ^{14}C which was recovered in hexose and in the products of respiration in the cortex and apex was roughly double that in the stele. Our results show that in both the cortex and the apical 6 mm the incoming [^{14}C]-sucrose was metabolized almost at once, part of it was converted to polysaccharide and the rest to hexose or respiratory intermediates.

Stele, cortex and apex all converted a significant proportion of the [^{14}C]-sucrose to polysaccharide. The proportion of the metabolized [^{14}C]-sucrose that was converted to polysaccharide was greater in the stele than in the cortex or apex. On our hypothesis, these results would be expected from the distribution of sucrose synthetase. The cortex and the apex differed from the stele in their much more rapid metabolism of the [^{14}C]-sucrose to hexose and respiratory intermediates. This difference correlates closely with the very high activity of acid invertase in the cortex and apex and its low activity in the stele. We conclude that when [^{14}C]-sucrose is supplied to pea roots under conditions approaching those in the intact plant, the distribution of label is consistent with the roles which we have proposed for sucrose synthetase, alkaline invertase and acid invertase.

If our view is correct then the problem arises of how sucrose, arriving in a root cell, is distributed between sucrose synthetase and the invertases. There is good evidence that both stuctural [6] and storage [7] polysaccharides are formed on or within intracellular membrane systems. Thus a possible means of regulating the interaction between sucrose synthetase and the invertases is location of sucrose synthetase within an organelle or on the endoplasmic reticulum. Therefore we investigated the intracellular location of sucrose synthetase.

We extracted samples of the apical 4 cm of the roots of 5-day-old pea seedlings by the gentle method previously shown to be suitable for the isolation of such fragile organelles as Golgi bodies [8] and proplastids [9]. We then fractionated the extracts by differential centrifugation and determined the distribution of sucrose synthetase and cytochrome oxidase. The distribution of cytochrome oxidase is used to indicate the extent to which our techniques preserved a well characterized organelle, the mitochondrion. Because the extractions were incomplete, the absolute activities of the two enzymes varied between extracts but the distribution of the activity between the different fractions showed little variation between experiments, and is represented by the experiment shown in Table 5. Examination of the different

Table 3. Distribution of ^{14}C in cortices of pea roots supplied with [U-^{14}C]-sucrose

	Distribution of ^{14}C							
Experiment no.	2		3		4		5	
	Pulse	Chase	Pulse	Chase	Pulse	Chase	Pulse	Chase
Incubation time (hr)	1	3	2	2	2	3	3	6
^{14}C recovered from cortices (dpm × 10^{-3})	17·28	19·49	36·95	58·75	50·27	51·69	109·8	101·4
% recovered ^{14}C found in:								
CO_2	—	2·9	—	3·5	—	3·8	—	6·5
Water-soluble substances	79·8	69·5	79·6	72·5	76·8	56·2	78·4	52·6
Acidic and basic components	20·1	24·6	26·1	34·1	24·8	25·2	20·8	26·0
Neutral components	59·6	44·9	53·5	38·4	52·1	31·1	57·6	26·6
Sucrose	26·3	5·7	24·6	13·0	20·1	3·9	13·8	1·4
Glucose	15·8	18·8	17·8	8·9	19·9	17·0	26·4	17·1
Fructose	12·0	13·3	7·6	10·1	10·1	5·2	13·6	2·9
Water-insoluble substances	20·2	27·6	20·4	23·9	23·2	39·9	21·6	40·8
Polysaccharides	—	—	—	—	15·3	29·9	15·0	27·6

Table 4. Distribution of ^{14}C in apical 6 mm of pea roots supplied with [U-^{14}C]-sucrose

	Distribution of ^{14}C								
Experiment no.	2			4		3		5	
	Pulse	Chase	Chase	Pulse	Chase	Pulse	Chase	Pulse	Chase
Incubation time (hr)	1	0·5	1	2	1	2	2	3	6
^{14}C recovered from apices (dpm × 10^{-3})	7.01	6·58	7·90	95·3	112·15	64·32	74·18	177·8	170·9
% recovered ^{14}C found in:									
CO_2	—	2·8	4·4	—	5·7	—	7·2	—	6·6
Water-soluble substances	75·5	61·7	54·5	65·4	49·3	68·2	43·8	63·0	42·0
Acidic and basic components	49·6	36·1	38·8	36·4	36·7	38·1	23·8	28·9	25·5
Neutral components	25·9	25·6	15·7	28·9	12·6	30·1	15·1	34·1	16·5
Sucrose	14·8	6·6	2·2	14·5	1·9	17·2	1·1	11·8	0·9
Glucose	6·1	8·2	5·8	7·0	4·2	6·4	5·6	13·0	9·0
Fructose	3·1	5·2	2·5	2·4	0·6	1·6	1·0	4·1	1·0
Water-insoluble substances	24·5	35·5	41·1	34·6	45·0	31·8	49·0	37·0	51·4
Polysaccharides	—	—	—	19·9	27·2	17·0	22·6	16·3	24·6

fractions in the electron microscope revealed that the sediment at 10800 *g* contained intact mitochondria and a few proplastids whilst that at 105000 *g* contained Golgi bodies and rough and smooth endoplasmic reticulum. There was no significant loss of cytochrome oxidase during the fractionation and over 80% of it was recovered in the fraction which contained the mitochondria. These results and the electron micrographs indicate that our extraction and fractionation of the pea roots gave adequate preservation and separation of most of the major membrane systems. The central feature of the results for pea roots is that none of the fractions which contained membranes or organelles contained a significant proportion of the sucrose synthetase activity. This was not due to loss of activity during the fractionation as we recovered nearly 90% of the activity originally present in the unfractionated homogenate and found over 80% in the supernatant obtained at 105000 *g*.

A more stringent test of whether sucrose synthetase is membrane-bound would be to examine its location in tissues in which massive conversion of translocated sucrose to polysaccharide is occurring. Considerable conversion of sucrose to starch occurs in young tubers of potato and in the developing spadix of *Arum maculatum* [10]. Thus we investigated whether sucrose synthetase in these organs was located in the amyloplasts. We extracted these tissues as we did the pea roots and sedimented the amyloplasts at 1000 *g*. Examination of the sediments in the electron microscope revealed that they consisted largely of amyloplasts and starch grains. In neither tissue did the amyloplast preparations contain a significant proportion of the total activity of sucrose synthetase.

In none of the tissues which we examined did we find any evidence that sucrose synthetase is membrane-bound. Our data indicate that this enzyme is in the cytoplasmic or soluble phase of plant cells. The techniques for fractionation of plant cells have not been developed to the point where it is possible to prove that an enzyme is soluble. None the less our data suggest that association of sucrose synthetase with a membrane system is not the means whereby interaction between this enzyme and invertase is regulated.

EXPERIMENTAL

Materials. Experiments with peas (*Pisum sativum* L. var. Kelvedon Wonder) were done with 5-day-old seedlings grown as described previously [3]. Spadices of *Arum maculatum* L., between stages β and γ as specified by Lance [10], were collected locally and used at once. Immature (6 × 4 cm) tubers of potato (*Solanum tuberosum* L.) were used.

Metabolism of [^{14}C]*-sucrose.* We prepared samples of 20 pea seedlings and removed the cortex from the regions of the roots 36–46 mm from the apices. Each sample of 20 seedlings was then put in a compartmented box so that [^{14}C]-sucrose could be applied locally and specifically to the bared regions of the steles. The details of the arrangement of the

Table 5. Distribution of sucrose synthetase in homogenates of plant tissues

Tissue	Fraction	Enzyme activity: Sucrose synthetase nkat/fraction	Sucrose synthetase % of total*	Cytochrome oxidase nkat/fraction	Cytochrome oxidase % of total*
Apical 4 cm of roots of 5-day-old peas	Unfractionated homogenate	22·2	100	239	100
	Sediment at 10800 *g*	0·9	4	193	81
	Sediment at 105000 *g*	none detected	0	51	21
	Supernatant at 105000 *g*	18·4	83	none detected	0
Developing tubers of potato	Unfractionated homogenate	6·1	100		
	Sediment at 1000 *g*	0·2	3		
	Supernatant at 1000 *g*	5·3	86		
Spadix of *Arum maculatum*	Unfractionated homogenate	13·9	100		
	Sediment at 1000 *g*	0·05	0·3		
	Supernatant at 1000 *g*	11·6	84		

* The activity of each fraction is expressed as a percentage of that present in the unfractionated homogenate.

seedlings in the boxes were the same as those for our previously described experiments [2] in which similar samples of seedlings were supplied with asymmetrically labelled [^{14}C]-sucrose. The pulse was started by adding 0·5 ml 0·02 M KH_2PO_4 (pH 5·2), which contained 10 μCi [U-^{14}C]-sucrose (0·3 mM), to a strip of filter paper which had been laid across the bared steles. The samples were then incubated at 25° in the dark. At the end of the pulse each sample of 20 plants was divided into two samples, each of 10 plants. Then each sample of 10 plants was treated as follows. The apical 6 mm of the roots were excised and the regions of the roots 6–24 mm from the apices were separated into stele and cortex as described before [11]. This material was sorted into samples of stele, cortex and apex. Thus the apical 24 mm of the roots of the original sample of 20 seedlings yielded duplicate samples of stele, duplicate samples of cortex, and duplicate samples of apices. One of each of these pairs of samples was killed at once and the other was put in 0·5 ml 0·02 M KH_2PO_4 (pH 5·2) in a 100 ml Erlenmeyer flask and incubated in the dark at 25°. This treatment is called the chase. During the chased respired $^{14}CO_2$ was collected in alkali in a centre well in the flask. We have described [2] the methods which we used for killing and extracting the samples and for determining the distribution of ^{14}C except for the preparation of the polysaccharide fraction. We obtained this by hydrolysing the alcohol-insoluble material and neutralizing the hydrolysate as described by Harris and Northcote [12]. Then we separated the sugars and uronic acids from the rest of the hydrolysate by paper electrophoresis according to Hanke and Northcote [13]. We eluted the sugars and uronic acids from the electrophoretogram with water and combined the eluates to give the polysaccharide fraction.

Extraction and assay of enzymes. For the assay of total sucrose synthetase activity tissues were extracted as before [4]. For the study of the intracellular distribution of this enzyme tissues were extracted as described by ap Rees *et al.* [9] in the following media: 17–25 g fr. wt of the apical 40 mm of pea roots in 0·167 M glycylglycine buffer (pH 7·4) which contained sucrose (0·4 M); 1–2 g fr. wt of the club of *Arum* spadix in 0·1 M Tris–HCl buffer (pH 7·6) which contained EDTA (20 mM), cysteine–HCl (20 mM), sodium diethyldithiocarbamate (20 mM) and sorbitol (0·4 M); 20 g fr. wt of potato tuber in the medium used for spadix except that the sorbitol was replaced by 0·4 M sucrose. The extracts were filtered through six layers of muslin to give the unfractionated homogenates which were centrifuged as follows: potato and *Arum* 1000 *g* for 5 min; pea 10800 *g* for 15 min followed by 105000 *g* for 60 min. The sediments were resuspended as follows: pea, 0·167 M glycylglycine (pH 7·4) that contained Triton X-100 (1% v/v); spadix, the extraction medium minus the sorbitol; potato, the extraction medium minus the sucrose but plus Triton X-100 (1%). Each of the fractions was then desalted and assayed for sucrose synthetase as described previously [4] and for cytochrome oxidase as described by Fritz and Beevers [14]. The techniques used for the examination of the different fractions in the electron microscope [9] and for the assay of ^{14}C[2] have been described. CO_2 production of the samples was measured manometrically.

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