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# SUCROSE METABOLISM IN STELE AND CORTEX ISOLATED FROM ROOTS OF *PISUM SATIVUM*

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**Key Word Index**—*Pisum sativum*; Leguminosae; root; sucrose metabolism; sucrose synthetase; sucrose phosphate synthetase.

**Abstract**—Stele and cortex were separated from the region 6–24 mm from the tip of roots of seedlings of *Pisum sativum* L. that had been grown in the dark for 5 days. The activities of sucrose synthetase (E.C. 2.4.1.13) and sucrose phosphate synthetase (E.C. 2.4.1.14) in extracts of stele were 34 and 5·9 nmol product formed/min per mg protein, respectively. The corresponding figures for extracts of cortex were 17 and 5·2. Appreciable labelling of sucrose occurred when samples of either stele or cortex were incubated in [$^{14}$C]glucose for 90 min. The labelling of sucrose after incubation of the tissues for 45 min in [$^{14}$C]glucose followed by 45 min in glucose suggested some turnover of sucrose in the cortex but none in the stele. These results are discussed in relation to the control of sucrose metabolism in the root.

## INTRODUCTION

WE HAVE compared the contents of sucrose, glucose and fructose with the activities of acid and of alkaline invertase during the differentiation of the pea root.[1] Our results are consistent with the view that variation in the maximum catalytic activity of acid invertase is one of the major factors that regulate sucrose content in the tips of pea roots. Variation in the activities of both sucrose synthetase (E.C. 2.4.1.13) and sucrose phosphate synthetase (E.C. 2.4.1.14) could also contribute to the control of sucrose metabolism in the root. This paper reports an investigation of this possibility in stele and cortex isolated from the region of pea roots 6–24 mm from the tip. We compared these two parts of the root because they differ markedly in their sugar content and acid invertase activity.[1] The stele, relative to the cortex, has a high content of sucrose, a low content of glucose and fructose, and a low activity of acid invertase. We carried out two types of experiment. Firstly, we determined the activities of the two synthetases in extracts of stele and cortex. The extraction procedure and assays that we used were based on those designed by Slack[2] and Hawker[3,4] to minimize interference from the products of phenol oxidation and the activities of phosphatases and invertases. Secondly, we investigated the relationship between our measurements of enzyme activities *in vitro* and sucrose synthesis and breakdown *in vivo*. We did this by determining the labelling of sucrose, glucose, and fructose in isolated steles and cortices that had been incubated in [$^{14}$C]glucose. The possibility that the labelling of the sugars represented turnover was examined in a pulse and chase experiment in which we measured the effects of incubation in glucose on the labelling pattern produced by [$^{14}$C]glucose. In these experiments the incubation periods were deliberately kept short so as to minimize the risk of complications arising from the development of induced respiration.[5,6]

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[1] R. L. LYNE and T. AP REES, *Phytochem.* **10**, 2593 (1971).
[2] C. R. SLACK, *Phytochem.* **5**, 397 (1966).
[3] J. S. HAWKER, *Biochem. J.* **105**, 943 (1967).
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[5] T. AP REES, *Austral. J. Biol. Sci.* **19**, 981 (1966).
[6] C. P. P. RICARDO and T. AP REES, *Phytochem.* **9**, 239 (1970).

## RESULTS

### *Measurements of Sucrose Synthetase and Sucrose Phosphate Synthetase*

Extracts of the apical 3 cm of pea roots were used to determine the pH optima of the above enzymes. Optimum activities of sucrose synthetase and sucrose phosphate synthetase were found at pH 8·5 and 6·5, respectively. In our assays the activities of both enzymes were constant for at least 1 hr and were negligible in the absence of UDPG. The activities of the enzymes in the extracts of stele and cortex are given in Table 1. Appreciable activities of both enzymes were found in extracts of both stele and cortex. In both types of extracts the activity of sucrose synthetase exceeded that of sucrose phosphate synthetase. The activity of sucrose synthetase in extracts of stele was double that in extracts of cortex. There was no significant difference in sucrose phosphate synthetase activity between the two extracts.

TABLE 1. ACTIVITIES OF SUCROSE SYNTHETASE AND SUCROSE PHOSPHATE SYNTHETASE IN EXTRACTS OF STELE AND CORTEX 6–24 MM FROM APEX OF PEA ROOT

| Enzyme | Activity* (nmol product formed/min/mg protein) | |
|---|---|---|
| | Stele | Cortex |
| Sucrose synthetase | 34 ± 2 (3) | 17 ± 3 (3) |
| Sucrose phosphate synthetase | 5·9 ± 0·2 (4) | 5·2 ± 0·5 (4) |

* Values are given as means ± S.E. The number of extracts assayed is given in parentheses.

### *Labelling of Sugars by* [U-$^{14}$C]*glucose*

Tables 2 and 3 show the labelling of sucrose, glucose, and fructose after incubation of stele and cortex in [U-$^{14}$C]glucose for 45 and 90 min. Also shown is the labelling of sugars after a pulse incubation in [U-$^{14}$C]glucose for 45 min followed by a chase incubation in unlabelled glucose for a further 45 min. We obtained these labelling patterns consistently with samples prepared from different batches of seedlings. The labelling patterns were determined by chromatography of samples of the neutral fractions of the water-soluble components of the tissues and media. We recovered, in sucrose, glucose, and fructose, between 85 and 98% of the $^{14}$C added to the chromatograms. Most of the outstanding $^{14}$C was recovered either from the origin of the chromatograms or from the region between the origin and sucrose. It is likely that this represented labelled oligosaccharides. We do not think that the patterns in Tables 2 and 3 were affected by losses during chromatography.

Steles and cortices absorbed appreciable amounts of the [$^{14}$C]glucose during the incubations. Uptake by both tissues appears to have been linear in relation to time. Both stele and cortex converted [$^{14}$C]glucose to sucrose. A much higher proportion of the absorbed [$^{14}$C]glucose was recovered in sucrose from cortex than from stele. The percentage of the absorbed [$^{14}$C]glucose that was recovered in sucrose increased during the second 45 min of the incubation. This increase was much more marked in the stele than in the cortex. The two parts of the root also differed in the behaviour of the [$^{14}$C]sucrose during the cold chase. In the stele the total $^{14}$C in sucrose increased during the cold chase and about a quarter of the labelled sucrose leaked into the medium. In the cortex the total $^{14}$C in sucrose declined during the cold chase and very little [$^{14}$C]sucrose was recovered from the medium.

TABLE 2. LABELLING OF SUGARS BY [U-$^{14}$C]GLUCOSE SUPPLIED TO STELES ISOLATED FROM TIPS OF PEA ROOTS

| | Uptake of [$^{14}$C]glucose | | $^{14}$C recovered, at the end of the incubation, in: | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Neutral fraction of aqueous extract | | Sucrose | | Glucose | | Fructose | |
| Origin of neutral fraction analysed | dpm per sample | % $^{14}$C supplied | dpm per sample | % $^{14}$C absorbed | dpm per sample | % $^{14}$C absorbed | dpm per sample | % $^{14}$C absorbed | dpm per sample | % $^{14}$C absorbed |
| Steles incubated in [$^{14}$C]glucose for 45 min | 140 000 | 7·3 | 11 800 | 8·4 | 4860 | 3·5 | 5500 | 3·9 | 490 | 0·3 |
| Steles incubated in [$^{14}$C]glucose for 90 min | 280 000 | 14·6 | 25 100 | 9·0 | 15 270 | 5·5 | 6100 | 2·2 | 600 | 0·2 |
| Steles incubated in [$^{14}$C]glucose for 45 min and then for 45 min in glucose | 133 000 | 6·9 | 8660 | 6·5 | 4130 | 3·1 | 2570 | 1·9 | 430 | 0·3 |
| Medium at end of above incubation in glucose | — | — | 5010 | 3·8 | 1550 | 1·2 | 2750 | 2·1 | 0 | 0 |

The changes in the labelling of sucrose during the cold chase, though small, were completely reproducible.

Intracellular glucose was labelled in both tissues, although the patterns of labelling differed. In the stele there was little increase in the labelling of glucose during the second 45 min whereas that in the cortex increased in proportion to the amount of [$^{14}$C]glucose absorbed. The two tissues also differed in their labelling of fructose. Only very small amounts of label were recovered in fructose in the stele. In the cortex fructose became increasingly labelled with time and showed a significant increase in labelling during the cold chase.

TABLE 3. LABELLING OF SUGARS BY [U-$^{14}$C]GLUCOSE SUPPLIED TO CORTICES ISOLATED FROM TIPS OF PEA ROOTS

| | Uptake of [$^{14}$C]glucose | | $^{14}$C recovered, at end of the incubation, in: | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Neutral fraction of aqueous extract | | Sucrose | | Glucose | | Fructose | |
| Origin of neutral fraction analysed | dpm per sample | % $^{14}$C supplied | dpm per sample | % $^{14}$C absorbed | dpm per sample | % $^{14}$C absorbed | dpm per sample | % $^{14}$C absorbed | dpm per sample | % $^{14}$C absorbed |
| Cortices incubated in [$^{14}$C]glucose for 45 min | 208 000 | 10·8 | 63 800 | 30·7 | 35 570 | 17·1 | 22 130 | 10·6 | 2770 | 1·3 |
| Cortices incubated in [$^{14}$C]glucose for 90 min | 400 000 | 20·8 | 129 000 | 32·2 | 79 370 | 19·8 | 39 870 | 10·0 | 7830 | 2·0 |
| Cortices incubated in [$^{14}$C]glucose for 45 min and then for 45 min in glucose | 195 000 | 10·1 | 57 200 | 29·4 | 30 270 | 15·5 | 20 730 | 10·6 | 3430 | 1·8 |
| Medium at end of above incubation in glucose | — | — | 5160 | 2·6 | 2100 | 1·1 | 2100 | 1·1 | 450 | 0·2 |

## DISCUSSION

We have published evidence that our dissection technique separates stele from cortex cleanly and reproducibly at the endodermis.[1,7] The reproducibility of the data in this paper

[7] W. J. L. WONG and T. AP REES, *Biochim. Biophys. Acta* **252**, 296 (1971).

augments this evidence. The evidence of linear uptake of [$^{14}$C]glucose in the present experiments, together with data on the respiration and on the metabolism of specifically labelled glucose by root segments[8] and isolated steles and cortices,[7] all indicate that the dissection and incubation had no obvious effect on carbohydrate metabolism. In particular it is unlikely that the results of the feeding experiments were affected by complications associated with induced respiration. We conclude that our data for isolated steles and cortices are relevant to the behaviour of these tissues in the root.

We argue that our assays of sucrose phosphate synthetase represent the activity of this enzyme because we measured sucrose phosphate formation from fructose-6-phosphate. Our results show that stele and cortex of pea root tips contain both sucrose synthetase and sucrose phosphate synthetase, and that both tissues synthesize [$^{14}$C]sucrose when incubated in [$^{14}$C]glucose. From the feeding experiments minimum estimates of the rates of [$^{14}$C]sucrose synthesis between 45 and 90 min can be made. These are 0·02 and 0·16 nmol [$^{14}$C]sucrose/min per mg protein for stele and cortex, respectively. Our data show that in stele and in cortex these rates could be sustained by either synthetase. In view of the evidence that sucrose phosphate synthetase is responsible for sucrose synthesis in both photosynthetic[9] and non-photosynthetic[10] tissues, we suggest that this enzyme is also responsible for the [$^{14}$C]sucrose synthesis found in our experiments. Tissues in which high activities of sucrose synthetase have been demonstrated are tissues that consume sucrose in considerable amounts.[10–13] We think that, in pea roots, sucrose synthetase contributes to sucrose breakdown as has been suggested for other tissues.[10–14] This implies that pea roots contain at least three enzymes, acid invertase, alkaline invertase and sucrose synthetase, that contribute to sucrose breakdown. We have discussed possible roles for the two invertases.[1] We suggest that sucrose synthetase contributes to sucrose breakdown in the cytoplasm where it might be particularly important in providing sugar nucleotides for the biosynthesis of polysaccharides. The higher activity of sucrose synthetase in the stele relative to that in the cortex may reflect either a greater need for sugar nucleotides during the differentiation of the stele or a greater need for enzymes catalysing sucrose breakdown in cells with low activity of acid invertase.

The abilities of stele and cortex to form sucrose from exogenous glucose, and their activities of the two synthetases, do not correlate with their sucrose content.[1] Thus we think it unlikely that the ability of the tissues to synthesize sucrose plays a direct role in determining sucrose content. The importance of sucrose synthesis in the root probably depends upon the extent to which sucrose turnover occurs and the extent to which the root cells obtain their carbon as hexose. Movement of sucrose out of the phloem, followed by hydrolysis by extra-cellular invertase, could provide the root cells with hexose. In the stele sucrose does not appear to turnover but sucrose synthesis could contribute to the sucrose content of any cells that are supplied with hexose. The role of sucrose synthesis in the cortical cells that contain relatively little sucrose is more difficult to decide. Here, either sucrose formation is quantitatively unimportant, or newly synthesized sucrose is broken down again. Our data suggest that the latter may be the explanation as there is evidence

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[11] R. PRESSEY, *Plant Physiol.* **44**, 759 (1969).
[12] G. A. MACLACHLAN, A. H. DATKO, J. ROLLIT and E. STOKES, *Phytochem.* **9**, 1023 (1970).
[13] D. P. DELMER and P. ALBERSHEIM, *Plant Physiol.* **45**, 782 (1970).
[14] W. Z. HASSID, *Ann. Rev. Plant Physiol.* **18**, 253 (1967).

that the label in sucrose declined during the cold chase whilst that in fructose rose. We have suggested previously that the high acid invertase activity of the cortex is at least partially responsible for its low content of sucrose.[1] In cells supplied with hexose from the free space, sucrose synthesis followed by rapid breakdown could occur if the synthesis took place in the cytoplasm and was followed by movement of the sucrose to the storage compartment that contained acid invertase. This suggestion is supported by work with other tissues. Sucrose synthesis from exogenous hexose has been demonstrated in a number of tissues that have high activity of acid invertase.[6,12,15,16] In two tissues[17,18] the sucrose formed from exogenous hexose has been shown to break down fairly rapidly, although the contributions to this breakdown of the phenomena associated with the development of induced respiration[5,6] have not been assessed.

## EXPERIMENTAL

*Material.* Peas (*Pisum sativum* L. var Kelvedon Wonder) were grown in the dark at 25° for 5 days and the root tips were harvested and dissected into stele and cortex as described previously.[1] The time between the dissection of the first root and both the preparation of the extracts and the addition of [$^{14}$C]glucose to the tissues was 3 hr.

*Assay of enzymes.* Samples of tissue (0·6–1·3 g fr. wt) were homogenized, first in a pestle and mortar, and then in an all-glass homogenizer in 1·5 vol. 100 mM Tris-HCl buffer (pH 7·6) that contained EDTA (20 mM), cysteine-HCl (20 mM) and sodium diethyl-dithiocarbamate (20 mM). Microscopic examination of these homogenates revealed very few unbroken cells. Homogenates were centrifuged at 35 000 *g* for 15 min and 1 ml of supernatant was then desalted by passage through a column (10 × 1·4 cm) of Sephadex G-25 (coarse) that had been washed with 10 mM Tris-HCl buffer (pH 7·0). All the above operations were carried out between 2 and 4°. Protein in the extracts was assayed as described previously.[1]

For the assay of sucrose synthetase the reaction mixture, at pH 8·5, contained in a total volume of 100 μl: 50 μl tissue extract, 3·0 μmol [U-$^{14}$C]fructose (0·02 μCi), 1·33 μmol UDPG, 5·0 μmol sucrose, and 3·33 μmol Tris-HCl. This mixture was incubated in small tubes at 25° for 30 min. The reaction was started by the addition of the [U-$^{14}$C]fructose and stopped by transferring the tubes, for 2 min, to a water-bath at 100°. Sucrose was isolated from 20 μl portions of the reaction mixture by paper chromatography on Whatman No. 3 paper in EtOAc–pyridine–$H_2O$ (8:2:1).

For the assay of sucrose phosphate synthetase the reaction mixture, at pH 6·5, contained in a total volume of 100 μl: 50 μl tissue extract, 1·5 μmol [U-$^{14}$C]fructose-6-phosphate (0·02 μCi), 1·33 μmol UDPG, 3·0 μmol EDTA, 1·67 μmol NaF, 3·33 μmol $K_2HPO_4$ and 3·33 μmol Tris-HCl. Incubations were carried out and stopped as described for sucrose synthetase. After denaturation of the extract, 20 μl portions of the reaction mixture were chromatographed as described above and the $^{14}$C in sucrose was determined. To the remainder of the reaction mixture we added 100 μl 50 mM glycine–NaOH buffer (pH 9·5) that contained 5·0 μmol $MgCl_2$, 5 μl alkaline phosphatase (Sigma Chemical Co., Type III-S) and 5 μl toluene. The tubes were then sealed and incubated overnight at 30°. Portions of the reaction mixture were then chromatographed and the $^{14}$C in sucrose was determined. The activity of sucrose phosphate synthetase is calculated from the difference in the $^{14}$C recovered in sucrose before and after treatment of the reaction mixture with alkaline phosphatase. All assays for both enzymes were corrected by values from blanks to which no UDPG had been added.

*Metabolism of* [$^{14}$C]*glucose.* Comparisons between stele and cortex are made only between tissues from the same batch of roots. Comparisons between the labelling at 45 and at 90 min, and between pulse and chase, are made only between replicate samples. Samples of stele (200 mg fr. wt) or cortex (250 mg fr. wt) were incubated at 25° in 2·5 ml 0·02 M $KH_2PO_4$ (pH 5·2) that contained 1 μCi [U-$^{14}$C]glucose at 0·3 mM. At the end of the incubation the medium was removed and the tissue was rinsed thrice, each time for 1 min with 5·0 ml portions of 0·02 M $KH_2PO_4$ (pH 5·2). In the pulse and chase, samples were incubated in [$^{14}$C]glucose for 45 min, then the medium was removed and the samples were rinsed as above, resuspended in 2·5 ml 0·02 M $KH_2PO_4$ (pH 5·2) that contained glucose at 0·3 mM, and incubated for 45 min. At the end of this cold chase the medium was removed and the samples were rinsed again. In all instances the rinsings were added to the media before the latter were analysed. Uptake of [$^{14}$C]glucose is given as

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[16] P. J. HARDY, *Austral. J. Biol. Sci.* **20,** 465 (1967).
[17] K. T. GLASZIOU, *Plant Physiol.* **36,** 175 (1961).
[18] A. L. KURSANOV, M. I. BROUCHENKO and E. P. BUTENKO, *Fiziol. Rast.* **14,** 813 (1967).

the difference between the $^{14}C$ supplied and that recovered in the medium and the rinsings. Evidence that the rinsing procedure removed the [$^{14}C$]glucose from the free space of the root is provided by an experiment in which 250 mg fr. wt of 3 mm segments, taken 6–24 mm from the root tip, were incubated in [$^{14}C$]glucose as above but for 15 min. At the end of this incubation the segments were given five 1-min rinses. The $^{14}C$ recovered in the medium and in the 5 successive rinses amounted to 1910, 242, 17·2, 2·14, 0·95 and 0·69 $\times$ $10^3$ dpm, respectively. A semi-logarithmic plot[19] of these results shows that 3 rinses sufficed to remove $^{14}C$ from the free space. All samples were killed and extracted with boiling 80% (v/v) aqueous EtOH. The water-soluble components of these extracts and the media at the end of the incubations were fractionated by ion-exchange chromatography.[15] The neutral fractions so obtained were analysed by paper chromatography in EtOAc–pyridine–$H_2O$ (8:2:1).

*Assay of* $^{14}C$. $^{14}C$ was assayed as described by Wong and ap Rees.[7] Compounds separated by paper chromatography were counted after elution from the paper with water.

*Acknowledgement*—R.L.L. thanks the Science Research Council for a research studentship.

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