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TRANSPORT OF SUGARS INTO THE SIEVE ELEMENTS OF WILLOW

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Abstract—An examination has been made of the labelled organic phosphates which are found in phloem exudate, obtained via severed aphid stylets, when radioactive sugars are applied to the cambial surface of bark strips of willow. It is proposed that the movement of sucrose into the sieve elements involves inversion by a free space invertase, resynthesis from the hexose moieties being accomplished via a UDP–UDP–glucose system in a metabolic compartment of the transport system. No evidence has been found for the involvement of a sucrose phosphate in the transport process.

INTRODUCTION

THE MAJOR proportion of the material translocated in the phloem consists of carbohydrate, and numerous analyses have shown that the predominant carbohydrate is sucrose, often with varying quantities of the raffinose family of oligosaccharides, raffinose, stachyose and verbascose.[1] However, other sugars can be moved into the sieve elements and transported,[2] the phloem showing an absolute preference for non-reducing as opposed to reducing sugars.

The movement of sugars into the sieve elements would appear to be an active process which requires metabolic energy,[3] but despite the investigations of Kursanov *et al.*[4] we know very little concerning the mechanism of sieve element loading. Hatch and Glasziou[5] using fructosyl-U-^{14}C sucrose have however shown that sucrose is not apparently inverted into its constituent hexose moieties during movement into the phloem of sugar cane. Even less is known about movement out of the sieve elements, though Zimmermann[6] has suggested that this is also an active process which may involve an α-D-galactosidase.

Much of the work on sugar uptake by plant cells has been carried out using leaf discs[7] or discs of storage tissue.[8,9] Hatch[10] came to the conclusion that sucrose phosphate was intimately concerned in the accumulation of sugars by sugar cane storage tissue. He demonstrated the presence of an enzyme (uridine diphosphate glucose-fructose-6-phosphate glucosyl transferase) which would synthesize sucrose phosphate, as well as isolating a compound with the properties of sucrose phosphate.

Using severed aphid stylets it is possible to tap the contents of a single sieve element, and therefore to follow the changes which occur in the sap during sugar loading. Some work

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[3] G. E. BARRIER and W. E. LOOMIS, *Plant Physiol.* **32,** 225 (1957).
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[5] M. D. HATCH and K. T. GLASZIOU, *Plant Physiol.* **39,** 180 (1964).
[6] M. H. ZIMMERMANN, *Recent Advances in Botany*, p. 1227, University of Toronto Press (1961).
[7] G. A. PENNELL and P. E. WEATHERLEY, *New Phytol.* **57,** 326 (1958).
[8] M. D. HATCH, J. A. SACHER and K. T. GLASZIOU, *Plant Physiol.* **38,** 338 (1963).
[9] M. D. HATCH and K. T. GLASZIOU, *Plant Physiol.* **38,** 344 (1963).
[10] M. D. HATCH, *Biochem. J.* **93,** 521 (1964).

using this technique has already been carried out.[11, 12] This demonstrated that considerable metabolic interconversions are undergone by sugars during their uptake into the sieve elements of bark strips of willow, and that two pathways may be involved; a direct one via the free space and companion cells, and an indirect one via the phloem parenchyma cells. The purpose of the work presented here is to further elucidate the processes involved in the sugar loading mechanism.

RESULTS AND DISCUSSION

Application of uniformly labelled ^{14}C-sucrose, glucose or fructose to bark strips leads to the appearance of activity in the stylet exudate not only in sucrose, and to a lesser degree in the hexoses, but also in organic phosphates.[11] Indeed within the first 2 hr of application, a greater proportion of the total activity may be present in the form of organic phosphates than in sucrose. It was therefore clearly desirable to determine the identity of these organic phosphates and to find out how the proportion of the total activity in each changed during the uptake process.

The identification of these compounds was carried out as follows. Stylet exudate samples from a number of bark strips to which U-^{14}C-sucrose had been applied were pooled. Samples were also taken from bark strips to which either U-^{14}C-glucose or U-^{14}C-fructose had been applied. These samples were then chromatogrammed in the *n*-butanol solvent in which the organic phosphates remain in the origin. Subsequent elution and chromatography of the organic phosphates in *n*-propanol–ammonia–water produced four active areas which contained over 90 per cent of the total activity of the organic phosphates, this pattern being found with all three sugars. The mobility ($R_{G\text{-}6\text{-}P}$ values) of these four peaks, together with the sugar phosphate and nucleotide standards with which they corresponded are shown in Table 1. It may be noted that peaks I and IV did not correspond to any of the standards employed, nor did Peak I have a mobility equivalent to any of those quoted by Bieleski and Young[13] for organic phosphates in this solvent.

Peaks II and III were then re-eluted and chromatogrammed in *n*-propyl acetate–formic acid–water. In this solvent the uridine nucleotides break down, whereas ATP and glucose-6-phosphate do not.[14] Peak II gave two active areas, Peak III produced three, none of which corresponded to either ATP, glucose-6-phosphate or any of the standards employed. However the two active areas from Peak II matched up with two of the three from Peak III, whilst the latter ran with the same mobility as the breakdown products from the UDP-glucose standard. Peak II would therefore seem to be UDP, Peak III, UDP-glucose.

Confirmation that none of the organic phosphate peaks separated in the *n*-propanol–ammonia solvent contained phosphates of sucrose, glucose or fructose was obtained by reacting these peaks with acid phosphatase, aliquots of the reaction mixture being chromatogrammed in *n*-butanol–acetic acid–water. No products of the reaction with acid phosphatase were produced which corresponded with standards of sucrose, glucose or fructose.

Experiments were then performed to determine the changes which occur in the activity of the organic phosphates during the process of ^{14}C-sugar uptake into the sieve elements of bark strips. Stylet exudate samples were taken at hourly intervals from the application of activity, the organic phosphates were separated from the other active components, then the

[11] J. FORD and A. J. PEEL, *J. Exptl. Bot.* **18,** 607 (1967).
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[13] R. L. BIELESKI and R. E. YOUNG, *Anal. Biochem.* **6,** 54 (1963).
[14] R. L. BIELESKI, *Plant Physiol.* **43,** 1297 (1968).

TABLE 1. THE MOBILITIES OF THE LABELLED ORGANIC PHOSPHATES OF THE STYLET EXUDATE AND THE SUGAR PHOSPHATE AND NUCLEOTIDE STANDARDS

Peak number	Mobility*
I	0
II	60
III	100
IV	160
Standards	
Glucose-1-phosphate	125
Glucose-6-phosphate	100
Fructose-1-phosphate	130
Fructose-6-phosphate	125
Fructose-1,6-phosphate	43
Adenosine-5-phosphate	129
Adenosine diphosphate	88
Adenosine triphosphate	59
Uridine diphosphoglucose	100

* $R_{G\text{-}6\text{-}P}$, glucose-6-phosphate = 100. in *n*-PpOH–NH_4OH–H_2O (6:3:1).

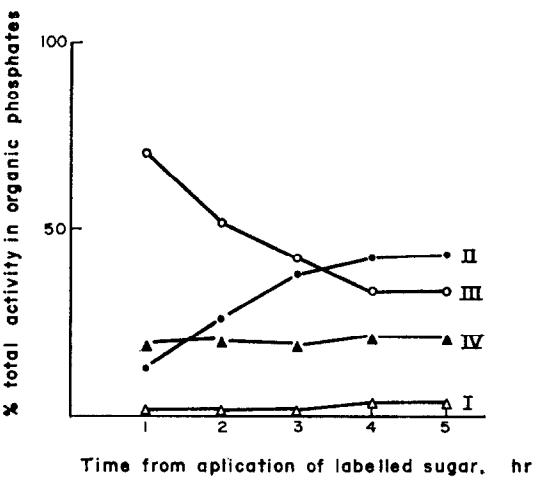

FIG. 1. CHANGES IN THE ACTIVITY OF THE ORGANIC PHOSPHATES OF THE STYLET EXUDATE DURING UPTAKE OF ^{14}C-LABELLED SUGAR INTO THE SIEVE ELEMENTS OF BARK STRIPS.

organic phosphates were chromatogrammed in the *n*-propanol–ammonia solvent. Figure 1 shows the results of such an experiment. The most striking feature is the relationship between Peaks II and III (UDP and UDP-glucose respectively) showing that UDPG becomes labelled very quickly. Its activity then declines whilst the UDP increases its share of the total activity in the organic phosphates.

Now what is the relevance of these observations to considerations of the mechanism of sugar uptake by the sieve elements of bark strips? Whilst we can draw no completely firm

conclusions, the scheme illustrated in Fig. 2, based on the proposals put forward by Peel and Ford[12] would seem to provide a basis for future work. The main feature of this scheme is that the plasmalemma forms a barrier to the free diffusion of sucrose. This conclusion is strengthened by the plasmolytic experiments of Currier *et al.*[15] Sucrose applied to a bark strip is hydrolysed by a free space invertase,[12] the resultant hexoses moving into the cytoplasm of the companion cell where they are resynthesized to sucrose via hexose phosphates and a UDP-UDP-glucose system. We have found no evidence that sucrose phosphate is implicated in this system, for it does not appear in the stylet exudate. However, this observation does not necessarily rule out the possibility that sucrose phosphate is involved in

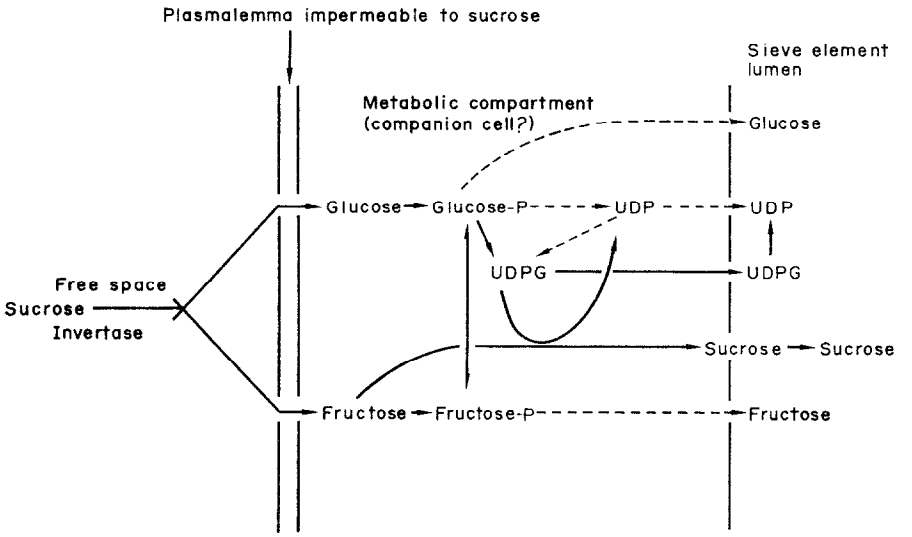

FIG. 2. SUGAR UPTAKE PROCESS BY SIEVE ELEMENTS OF BARK STRIPS

sugar accumulation, for the sieve elements contain acid phosphatase.[16] Also, we have been unable to detect hexose phosphates in stylet exudate, in spite of the fact that these must be involved in the accumulation process. The turnover of labelled hexose phosphates may occur at a fast rate, and any that enter the sieve elements may be broken down by phosphatase enzymes.

As mentioned previously, Peel and Ford[12] on the basis of the distribution of activity in stylet exudate, suggested that two pathways may operate in bark strips for the transport of sugars into the sieve elements. The experiments described so far have been concerned only with the situation prevailing on the direct pathway. The indirect pathway, on which sugars are moved into the sieve elements from the storage parenchyma of the stem or the mesophyll cells of the leaf, is clearly of greater importance in the normal functioning of the plant. However, the direct pathway is much more amenable to investigation than is the indirect, owing to the complexity of the latter pathway. Experiments with bark strips, in which movement of labelled sugars was taking place on the indirect pathway, and also experiments with whole plants where ^{14}C-sugars were being moved into the sieve elements from the mesophyll cells of the leaf after $^{14}CO_2$ application to the leaf, have demonstrated

[15] H. B. CURRIER, K. ESAU and V. I. CHEADLE, *Am. J. Bot.* **42,** 68 (1955).
[16] H. H. LESTER and R. F. EVERT, *Planta* **65,** 180 (1965).

that a small (5%) proportion of the total activity in stylet exudate is present in organic phosphates. Moreover these organic phosphates would seem to be the same as those which are produced on the direct pathway, since their mobility in the *n*-propanol–ammonia solvent are the same. It is probable therefore that the loading mechanisms for sucrose on the two pathways are also fundamentally the same.

EXPERIMENTAL

Plant material. Bark strips and whole plants were both prepared from mature (2–4 yr old) stems of the common osier (*Salix viminalis* L.). The bark strip technique, which allows the application of solutions to the cambial surface of the bark, is described in detail by Weatherley *et al.*[17] The whole plants consisted of 50 cm lengths of stem which were rooted in potting compost and allowed to develop a single leafy shoot at the apex.

The aphid stylet technique. Sieve-tube exudate was obtained from both bark strips and mature stems of the whole plants via the severed stylets of the aphid *Tuberolachnus salignus* (Gmelin). The exudate was collected in 2 μl capillaries prior to being spotted onto strips (3·0 × 50·0 cm) of chromatography paper (Whatman No. 1).

Labelled compounds. Sucrose-U-^{14}C (463 mc/mM), glucose-U-^{14}C (230 mc/mM) fructose (210 mc/mM) and ^{14}C Na_2CO_3 (1·0 mc/mM) were obtained from commercial sources. Aqueous solutions of the labelled sugars were introduced onto the cambial surface of the bark strips, 5 μc in 3 ml of solution being employed. In the whole plant experiments $^{14}CO_2$ (50 μCi) was introduced into a flask surrounding the leaves. This was released from the $Na_2{}^{14}C$ with 10% lactic acid.

Chromatography. Initial separation of the components of the sieve-tube exudate was carried out by descending paper chromatography in *n*-BuOH–HOAC–H_2O (6:1:1). The two solvents used for the separation of the organic phosphates were *n*-PrOH–NH_4OH (sp. gr. 0·88)–H_2O (6:3:1) and *n*-PrOAc–HCOOH (90%)–H_2O (11:5:3). Location of the active compounds on the chromatogram strips was achieved by scanning the strips with a windowless chromatograph scanner.

Inactive organic phosphate standards were detected by spraying the appropriate strips with 1 g ammonium molybdate, 4·2 ml $HClO_4$ (70%), and 0·8 ml conc. HCl in 95 ml H_2O. After drying at room temp. the spots were developed by irradiating with UV light for a few minutes.

Assay of radioactivity. The active compounds were first eluted from the chromatogram strips into scintillation vials and after the addition of a toluene phosphor were counted in a liquid scintillation spectrometer.

[17] P. E. Weatherley, A. J. Peel and G. P. Hill. *J. Exptl. Bot.* **10**, 1 (1959).