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SHORT COMMUNICATION

METABOLISM AND TRANSPORT OF ^{14}C-LABELLED GLUTAMIC AND ASPARTIC ACIDS IN THE PHLOEM OF WILLOW

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Abstract-Uniformly labelled ^{14}C-glutamic and aspartic acids were applied to bark strips of willow, sieve tube exudate being obtained via severed aphid stylets. The label appeared in the amino acids in the stylet exudate within 1 hr of their application. However both amino acids were extensively metabolized, and within 2-3 hr from application the greatest proportion of the activity in the stylet exudate was present in sucrose.

INTRODUCTION

IT HAS been known for a number of years that nitrogenous compounds can be transported in the phloem.[1] Using labelled amino acids, applied to the petioles, Nelson and Gorham[2] were able to follow their translocation and metabolism in stems of soyabean. They concluded that the amino acids they employed were translocated without undergoing metabolic change. Further studies with soyabean,[3] in which $^{14}CO_2$ was supplied to the leaves, demonstrated the subsequent presence of labelled amino acids in the stem, though it was not possible to ascertain whether they had been translocated from the leaves. Work with isolated vascular bundles from leaves[4] has shown that the uptake of amino acids by these tissues is an active process which is highly selective.

Sieve tube exudate from willow contains a number of amino acids,[5,6] two of which, glutamic and aspartic acids, are present throughout the year together with the amides glutamine and asparagine. The other free amino acids are only found in the autumn during the period of leaf senescence. It seemed reasonable therefore, in this initial study of the uptake and metabolism of amino acids by phloem, to use glutamic and aspartic acids.

RESULTS AND DISCUSSION

Uniformly labelled ^{14}C-L-glutamic acid (5 μCi in 3 ml of distilled water) was applied to the cambial surface of a bark strip on which an exuding stylet had been established. A continuous collection of the stylet exudate was then made over a period of 10 hr, the exudate being subsequently chromatogrammed in the phenol-water solvent, together with amino acid, amide and sugar standards. Three radioactive areas were found in the stylet exudate one of which (Peak II) corresponded with both sucrose and asparagine, whilst a second (Peak III) ran with the same R_f as glutamic acid (Table 1). Elution of the active areas from

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TABLE 1. THE R_f VALUES OF THE LABELLED COMPOUNDS FOUND IN THE STYLET EXUDATE WHEN U-^{14}C-L-GLUTAMICACIDWASAPPLIEDTOABARK STRIP

Compound	R_f in Phenol-Water (4:1)	R_f in n-Butanol-acetic acid-water (6:1:1)
Peak I	0	0
Peak II	0·40	0.38
Peak III	0·30	0·75
Peak IV	0.60	
Aspartic acid	0.19	0·50
Glutamic acid	0·30	0·75
Asparagine	0.40	0·23
Glutamine	0·57	0.41
Sucrose	0·40	0.35
Glucose	0·38	0·71
Fructose	0·51	0.95

the chromatogram strip followed by chromatography in n-butanol-acetic acid-water showed that Peak II now ran with the same R_f as sucrose, Peak III once again corresponding to glutamic acid (Table 1). Confirmation that Peak II was indeed sucrose was obtained by reacting it with yeast invertase, followed by chromatography of the reaction mixture in the n-butanol-acetic acid solvent. Two radioactive areas were produced as a result of this treatment, one corresponding to a glucose standard, the other to fructose. Peak I remained on the origin of the chromatogram in both solvents and did not match up with any of the standards employed.

A further series of experiments was then performed in which collections of stylet exudate were made at hourly intervals after the application of the labelled glutamic acid. These

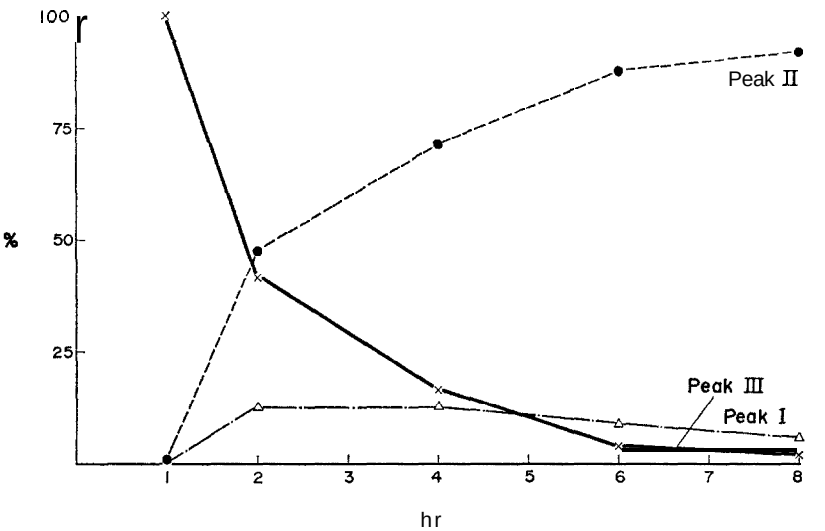

FIG. 1. CHANGESINTHEACTIVITYOFTHELABELLED COMPOUNDS IN THE STYLET EXUDATE AFTER THE APPLICATION OF U-^{14}C-L GLUTAMIC ACID TO A BARK STRIP.

showed that labelled glutamic acid was always present in the stylet exudate within 1 hr from the application of activity to the strip. However labelled sucrose also appeared in the exudate within 2 hr, and both this and the unknown Peak I subsequently increased their proportion of the total activity in the stylet exudate, whilst that in glutamic acid declined with increasing time (Fig. 1).

In certain of the experiments with glutamic acid an extract of the bark was made at the termination of the experiment with hot 70 % ethanol. This revealed a fourth active area to be present, running with an R_f 0·60 in the phenol/water solvent, and which corresponded with the glutamine standard.

The experiments in which 5 μCi of uniformly labelled ^{14}C-L-aspartic acid was applied to bark strips, produced results which were very comparable to those previously described using L-glutamic acid. Three active peaks were again found in the stylet exudate samples within 2 hr of the application of the labelled aspartic acid. One of these peaks was identified as aspartic acid, another as sucrose, whilst the third corresponded chromatographically with the unidentified peak (I) from the glutamic acid experiments. Also, the proportion of the total activity in the stylet exudate samples which was present in sucrose and the unidentified peak increased with time whilst that in the aspartic acid fraction decreased.

It is clear from the data presented, that investigations on the transport of glutamic and aspartic acids into the sieve elements of willow is complicated by extensive metabolism of these compounds. The fact that sucrose was produced from these amino acids was unexpected, though there are a number of reports in the literature[7–9] of the formation of sugars from amino acids. Several problems are raised by these results, not the least of which are those concerning the origin of the amino acids found in stylet exudate and the control of their concentrations.

EXPERIMENTAL

Plant material. Mature (2–4 yr old) stems of the common osier (***Salix viminalis*** L.), in the form of bark strips, were used in all experiments. The bark strip technique, which is described more fully by Weatherley ***et al.***,[10] enables solutions to be introduced onto the cambial surface of the bark.

Aphid stylet technique. Severed stylets of the aphid ***Tuberolachnus salignus*** (Gmelin) were used to obtain sieve tube exudate from the bark strips. The exudate was collected in 2 μl capillaries and then spotted onto strips of Whatman No. 1 chromatography paper (3·0 x 50 cm).

Labelled compounds. Commercially prepared L-glutamic acid-U-^{14}C (> 225 mCi/mM) and L-aspartic acid-U-^{14}C (> 229 mCi/mM) were used as aqueous solutions containing 5 μCi/3 ml distilled water. Three millilitres aliquots of these solutions were applied to the cambial surface of bark strips immediately after stylectomy.

Chromatography. Two solvents were employed for the separation of the amino acids-phenol-water" (80: 20, w/v) and n-butanol-acetic acid-water (6: 1: 1). The latter solvent also served to separate the three sugars encountered. Activity was located on the chromatogram strips with the aid of an actigraph III chromatogram scanner.

Sucrose ***hydrolysis.*** The sucrose containing solutions were treated with 5 μl of yeast invertase concentrate (BDH) for several hours. Separation of the hexoses liberated in this way was achieved by chromatography in the n-butanol solvent.

Assay ofradioactivity. A liquid scintillation spectrometer was used to count all samples.

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