SELECTIVE AND STEREO-SPECIFIC ABSORPTION OF VARIOUS AMINO ACIDS DURING XYLEM TRANSLOCATION IN TOMATO STEMS

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SUMMARY

Solutions of ^{14}C-labelled amino acids were allowed to percolate through the xylem vessels of tomato stem parts. The amounts absorbed by the stem tissues were determined by analyses of the collected fluid.

The protein amino-acids glycine and L-α-alanine were strongly retained by the stem tissues and partly metabolized. Hardly or no absorption, however, was found for either the protein amino-acids L-proline and L-glutamic acid, or for the "unnatural" amino-acids D-α-alanine, D-glutamic acid and α-aminoisobutyric acid.

It is concluded that during xylem translocation of amino-acids a selective uptake may occur by binding sites from which at least some have a stereo-specific affinity for these compounds.

1. INTRODUCTION

The occurrence of amino-acids and amides in the xylem bleeding sap of the root system of the tomato plant is caused mainly by the presence of ammonium ions in the nutrient solution.

If ammonium is the sole source of nitrogen for tomato growth most of the N required for leaf growth is exported by the roots in the form of glutamine, glutamic acid, the leucines, proline, gamma-aminobutyric acid, asparagine, aspartic acid and small amounts of the other common amino acids, especially α-alanine and glycine.

The question arises along which pathways these amino acids move upward to the leaves in the intact plant. From their presence in the xylem exudate one would be inclined to believe that the xylem sap stream, i.e. the transpiration stream, would be the normal translocation pathway of these amino compounds.

Some previous observations led to the suggestion that amino-acids may be strongly absorbed by living cells during xylem translocation (VAN DIE 1961). The remarkable minor role of α-alanine and glycine in the bleeding sap (VAN DIE 1958) and the hypothesis of JOY (1962) about different mechanisms of translocation for protein amino-acids and non-protein amino-acids made it worthwhile to investigate the behaviour of various representatives of metabolically different groups of amino-acids during xylem translocation.

The present paper describes a number of experiments in which ^{14}C-labelled amino-acids were allowed to percolate through the xylem vessels of a 10–12 cm length of an excised tomato stem. As "normal" protein amino-acids were used: glycine, L-proline, L-α-alanine and L-glutamic acid; as "unnatural" protein amino-acids D-α-alanine and D-glutamic acid. α-Aminoisobutyric acid was used as a "non-naturally occurring" amino-acid.

2. MATERIALS AND METHODS

Tomato plants (Ailsa Craig) in the flowering and fruiting stage, cultivated on nutrient solution (HOAGLAND & BROYER 1936), were used. They were topped above the second or third flower cluster after which several "thefts" developed. One or two hours before the experiments started, the plants were covered with a plastic bag to get tissues with a high degree of water saturation. The leaves were removed and after the appearance of drops of exudate at the cut ends of the petioles, "theft" internodes with a length of about 10–12 cm were cut off, quickly brought into a large beaker and left under distilled water for a few minutes. A short piece of silicone rubber tubing was fitted with care at the lower end of the stem part, in such a way that water present in the tubing could not leak between tubing and stem, but also without visible damage to the stem end.

Stem parts with water-filled tubing were clamped in a vertical position with the tubing at the top. The stem parts were blotted with lens paper. When within approximately 15 minutes the formation of a drop of water could be observed at the lower end, the stem part could be used for the experiment.

In some experiments the selected stem part was brought into a somewhat shorter piece of glas tubing of 2 cm width provided with two small side tubings for air inlet and outlet. The stem was fixed practically air tight at the top and bottom part of the glas tubing with the aid of para-rubber caps in which holes with a diameter somewhat smaller than that of the stem parts were punched. The air inlet was connected with a source of CO_2-free air and the outlet with a Recording CO_2-Analyser (Wösthof G. M. B. H., Bochum, W. Germany). The sodium hydroxide solution (0.01 Molar) used in this analyser, which has partly reacted with the CO_2 and which is continuously flowing out of it, was collected with a fraction collector with a rate of 0.83 ml.min^{-1}. Each fraction contained the $^{14}CO_2$ produced in 5.0 minutes; it was precipitated as $BaCO_3$, plated, and counted with a G. M. tube (1.5 mg.cm^{-2}). In this way both the CO_2 content of the gas stream that flowed along the stem part, and the amount of $^{14}CO_2$ it contained, could be continuously recorded and measured.

The water in the silicone rubber tubing was pipetted off and immediately replaced by an exactly known volume (0.4–0.5 ml) of a solution of two amino-acids (both 0.005 molar and containing 0.5 μc of carbon-14). The following solutions were used: (1) α-aminoisobutyric acid-1-^{14}C, and glycine-^{14}C (U), (2) L-proline-^{14}C (U) and L-α-alanine-1-^{14}C, (3) L-proline-^{14}C (U) and D-α-alanine-1-^{14}C, (4) L-glutamic acid-^{14}C (U) and D-α-alanine-1-^{14}C, (5) D-glutamic acid-1-^{14}C and L-α-alanine-1-^{14}C. Both the α-alanine and the glutamic acid isomers were obtained from the California Corporation for Biochemical Research, the other amino-acids from the Radiochemical Centre, Amersham, Great Britain.

At regular measured intervals drops could be collected from the free end of the stem part. Their volume was weighed and aliquots of them were spotted onto paper strips (Whatman No. 1). These were subsequently chromatographed one-dimensionally, using phenol-water-ammonia (80:15:1) as a solvent, dried

at room temperature for at least 48 hours, and semi-automatically scanned at both sides, with a velocity of 30 cm.hr^{-1}.

As soon as the radioactive solution had completely entered the stem part, small amounts of distilled water were brought the rubber tubing. This washing procedure was continued until no further activity could be collected at the lower end of the stem part.

Aliquots of the original amino-acid mixture were chromatographed and scanned in the same way, so by simple calculation it could be learned how much of the supplied amino acid was recovered in the series of collected drops.

During all experiments the stem parts were kept in the dark at a temperature of 18–20 °C.

3. RESULTS

1. In order to ascertain the pathway of the solutes through the stem parts in the present experiments, a solution of indian ink was allowed to percolate. After having passed a 10 cm stem part, its track could be observed by means of transverse and lengthwise cuts. The ink appeared to have moved only through the xylem vessels.

2. As might be expected, the percentage of amino-acid retained by the stem tissues in a percolation experiment appeared to increase with decreasing rate of movement of the solution through the xylem vessels. This is demonstrated for glycine (*fig. 1*) where the rates of movement in two experiments (volume I and II) are compared with the respective curves of the recovery percentages. It is obvious from these experiments that in all experiments where the behaviour of two amino-acid species is compared, differences in flow rate must be avoided. As, however, the rate of flow is difficult to control, comparisons between different amino-acids were always carried out by using mixtures of two easily separable components.

3. *Fig. 2* and *2a* show the differences in recovery rate between glycine and α-aminoisobutyric acid, a non-naturally-occurring amino-acid. The latter proves to be retained in the stem to a considerably smaller degree than the former.

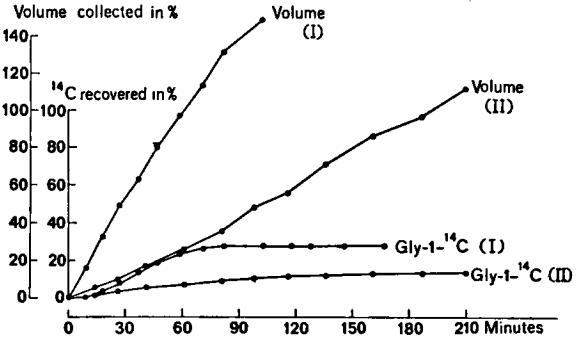

Fig. 1.
A comparison of the rates of percolation of 0.40 ml of 0.005 Molar glycine through a stem part in two experiments (I and II).

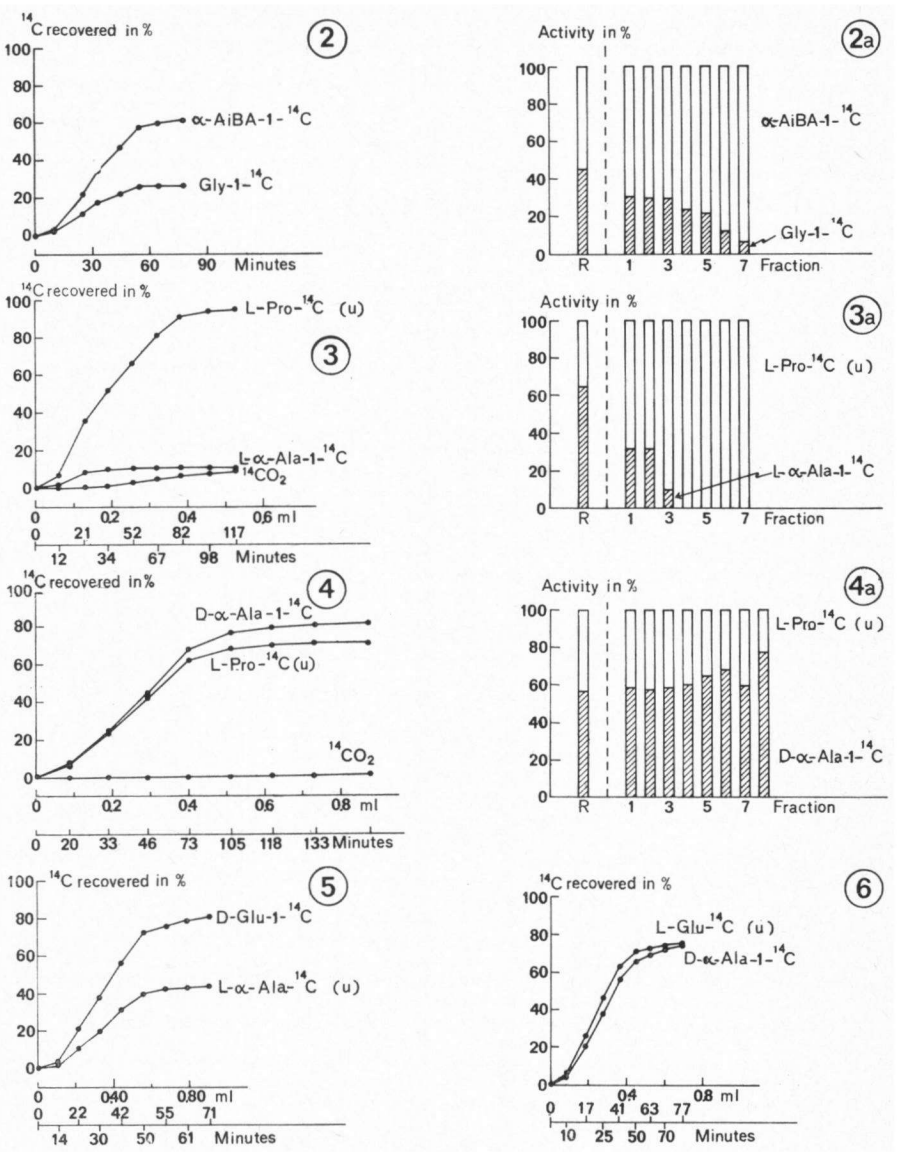

Fig. 2, 3, 4, 5 and 6. Differences in recovery rate of various amino acids when applied in pairs to stem parts.

Fig. 2a, 3a and 4a. The relative amounts of the amino acids in the various consecutive drops collected during the experiments. The amounts are expressed as percentages of the total activity of each drop. The relative amounts of the two components in the applied solutions are designated as „R".

Apparently a relation exists between the molecular structure of the amino-acids and the ability of tomato tissues to remove them from the xylem stream.

4. A similar type of experiment is shown in *fig.3* and *3a*. Here L-proline and L-α-alanine, both protein amino acids, are compared. During the passage of the amino-acid mixture through the stem part only the alanine is strongly retained. The proline is almost quantitatively recovered in the percolate. Moreover, a considerable amount of $^{14}CO_2$ originating from the carboxylic acid group of the α-alanine, evolved from the stem parts during the experiment (8–9% of the applied α-alanine activity).

5. The behaviour of a mixture of L-proline and D-α-alanine was also investigated. *Fig.4* and *4a* demonstrate the results. Neither L-proline nor D-alanine show much uptake by the stem tissues. Only traces of $^{14}CO_2$ could be recovered (less than 0.1 per cent of total applied activity). It is evident that the binding of α-alanine to the tissues surrounding the xylem vessels is stereo-specific and represents real uptake as it is followed by the decarboxylation of the L-form of the amino acid.

6. The processes involved in amino acid uptake during xylem transport appeared to be rather complex as neither the normal L-form, nor the "unnatural" D-form of glutamic acid showed an appreciable loss during percolation experiments. The *figs.5* and *6* show the results of experiments with these substances as compared with D- and L-α-alanine.

4. DISCUSSION

Little is known about possible substrate specificity of the amino acid uptake and transport mechanism of plant cells, in contrast with the existence of such a specificity in some mammalian tissues (e.g. BLASBERG & LAJTHA 1965).

Results obtained by EVERED & SNELL (1965) did not indicate the existence of transport processes in carrot root tissues that would be specific for amino acids occurring in plant proteins or in plant cells in general. Effects of D-amino acids on plant metabolism are demonstrated by ELLIS (1964) and by VALDOVINOS & MUIR (1965).

The experiments described in the present study clearly show that L-α-alanine is strongly retained by stem tissues during its movement through the xylem vessels, in contrast with the D-isomer. The L-form apparently also enters the living cells as it becomes decarboxylated to a considerable extent during the time of the experiments.

From experiments with leaves of *Atropa* and *Prunus* CHARLES (1953) concluded that the xylem elements of the petioles are negatively charged. Acidic substances, as penicillin and acid fuchsine appeared to enter the xylem with ease, while basic molecules as streptomycin and methylene blue showed a very slow petiolar uptake.

An assumed negative charge of the xylem walls of tomato plants would certainly have a favourable effect on the absorption of amphoteric molecules such

as glycine and α-alanine, while more acidic substances as the glutamic acid isomers would have a lower affinity to these walls.

Adsorption to the xylem walls certainly is a prerequisite for real uptake into surrounding cells. Apparently this uptake process proper takes place by a mechanism with distinct substrate specificity; even within the group of the neutral protein amino acids: L-α-alanine and glycine are absorbed, but not L-proline.

For the time being, it seems probable that both a negative charge of the vessel walls and a substrate specificity of the sites of entry of the amino acids into the living xylem cells belong to the factors that are responsible for the phenomena observed in the present study. Further investigations are needed before a more clear-cut picture can be presented.

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