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THE ABSORPTION OF L-α-ALANINE AND L-α-AMINO-ISO-BUTYRIC ACID DURING THEIR MOVEMENT THROUGH THE XYLEM VESSELS OF TOMATO STEM SEGMENTS

A. J. E. VAN BEL

Botanisch Laboratorium, Utrecht

SUMMARY

Two labelled amino acids (1 ml, 0.005 M), L-α-alanine and L-α-amino-iso-butyric acid were allowed to perfuse separately through isolated stem segments of about 12 cm length. After a rapidly increasing initial absorption a steady-state absorption was established, from which for each amino acid the fractional rate of escape per unit stem length could be calculated (*K in mm^{-1}). The values found could be confirmed by measuring the radioactivity in one-cm pieces of the stem segment used. *K-alanine was found to be about 8 × *K-α-amino-iso-butyric acid.

Influences of transpiration and flow rate through the xylem vessels on the *K-values could be demonstrated. A provisional model for amino acid absorption from the xylem vessel is presented.

1. INTRODUCTION

Amino acids are translocated in apical direction through the xylem vessels after being produced by the roots of *Lycopersicon esculentum* (VAN DIE 1963). On their way up a part of them is retained in the stem tissues. This might be explained by an initial binding to the negatively charged ionic groups in the cell walls assumed by CHARLES (1953). But also metabolizing cells around the xylem vessels might play a role as suggested by BIDDULPH et al. (1961) and JACOBY (1965). Together it results in a two-phasic absorption system, BIDDULPH et al. (1961) and HILL-COTTINGHAM & LLOYD-JONES (1968).

For amino acids an absorption selectivity has been shown by VAN DIE & VONK (1967). In their experiments with calcium transport through the xylem, BELL & BIDDULPH (1961) and HEINE (1970) found exponential accumulation gradients. In phloem translocation such gradients are known both for inorganic solutes (BIDDULPH & CORY 1957, SPANNER & PREBBLE 1962) and for assimilates (EVANS et al. 1963, CLAUSS et al. 1964, HO and PEEL 1969, WHITTLE 1970). In general these results are explained referring to the calculations of HORWITZ (1958).

The present paper describes a more detailed investigation into the absorption of amino acids from the xylem sap stream. In variation with earlier experiments (VAN DIE & VONK 1967) the present experiments are based upon the perfusion of single amino acids, i.e. ^{14}C-alanine or ^{14}C-α-amino-iso-butyric acid through isolated segments of tomato stems. From the logarithmic accumulation gradients found, the absorption coefficients of the amino acids used could be calculated. The results are briefly discussed.

2. MATERIAL AND METHODS

Tomato plants, cv. All Round, were grown in water cultures (HOAGLAND & BROYER 1936). When the plants were 50 cm high, the top was removed and "thefts" emerged out of the leaf-axils. After having reached a length of 10–20 cm the thefts were cut off under water. For one hour the stem segment was kept in water of 20 °C in order to saturate the cells. Then a well-closing silicone tube was carefully fitted around the morphological bottom of the tissues involved.

After being taken from the water a plastic sheet was immediately wrapped around the stem segment. A shorter silicone tube was fitted around the upper part and the stem segment was fixed in a vertical position, the morphological bottom on top. Initially 0.1 ml distilled water was allowed to perfuse through the stem segment followed by 1.0 ml 0.005 M ^{14}C-labelled amino acid solution (L-α-alanine (U) or L-α-amino-iso-butyric acid (U)). At regular intervals drops of the percolated solution could be recovered with a 50 µl microcap. When the solution had passed the stem segment 0.5 ml distilled water was put into the silicone tube. The recollection of the 50 µl drops of the percolate was continued till virtually devoid of radioactivity.

Each of the recovered drops was solved in 15 ml scintillation liquid and measured in a liquid scintillation spectrometer (Packard). One litre of counting solution was made up of 5.3 g PPO-POPOP (Packard Premix M), dissolved in a mixture of 750 ml toluene and 250 ml methanol.

The stem segment was frozen in liquid nitrogen and, lying on carbon dioxide ice, divided into pieces of 10 mm length by a thin iron-saw. The sections were separately ground in 0.5 ml 75% ethanol and the soluble radioactivity of each was measured after dissolving 0.1 ml extract in 15 ml of scintillation liquid.

The labelled amino acids were obtained from the Radiochemical Centre, Amersham, U.K.

3. SOME THEORETICAL CONSIDERATIONS ON THE MOVEMENT OF SOLUTES THROUGH XYLEM VESSELS

If a xylem vessel through which a solution is passing at a constant rate is regarded as a pipe with a wall permeable to solute and solvent, then a linear relationship between the logarithm of the concentration of the solute and the distance of movement from the point of entry into the stem may be expected. This is true for the amount of solute remaining in the xylem vessel or for that which escapes irreversibly from it into surrounding cell walls and cells. The slope of the curve will be directly related to the degree of leakage, regardless of the mechanisms of escape. Mathematical treatments of these aspects of translocation have been given by HORWITZ (1958). The escape of the solute molecules may be of a physical kind, viz. diffusion out of the moving solution into the free space arround it or exchange with charged constituents along the translocation pathway. It may also have a more chemical or metabolic character,

for example active absorption from the free space into the metabolic compartments of adjacent cells.

According to HORWITZ (1958) logarithmic removal of solutes from a vessel may expressed as

$$C_p = C_o \cdot e - \frac{KL}{A_pV} \text{ or } \frac{dC_p}{dL} = -\frac{K}{A_pV} \cdot C_p \quad (1)$$

in which
C_o is the radioactivity in cpm at the top of the vessel
C_p is the radioactivity in cpm at the lower end of the vessel
L is the length of the stem in mm
A_p is the transverse-sectional area of the vessel (mm^2)
V is the velocity of flow ($mm.hr^{-1}$)
K is a constant characteristic for the rate of escape of the solute from the vessel. It has the dimensions of a diffusion constant or a first-order chemical reaction-rate constant ($mm^2.hr^{-1}$).

$$K = \ln \frac{C_o}{C_p} \cdot A_pV/L \text{ or } K = \frac{2.303 \log C_o/C_p}{L} \cdot A_pV \quad (2)$$

In the tomato stem segments many vessels are involved in the conduction of the amino acid solution which percolates through them. A_p, therefore, is the sum of the transverse sectional areas of these vessels. Both A_p and V (the average velocity of flow through the vessels) are unknown, but their product A_pV is the rate of flow through the stem segment ($mm^3.hr^{-1}$). It can be measured accurately. In the present experiments use has been made of another constant *K, defined as

$$*K = \frac{K}{A_pV} \text{ or } *K = \frac{\ln C_o/C_p}{L} \quad (3)$$

It has the dimensions of mm^{-1} and represents the fractional rate of escape of the solute from the vessel per unit length of the stem.

4. RESULTS

4.1 The absorption of L-α-alanine and L-α-amino-iso-butyric acid

Starting from the assumption that a free space absorption by the xylem vessel walls and neighbouring cell walls is followed by an inner space absorption – whether or not under metabolic control – a relatively large volume (1.0 ml) of the amino acid (0.005 M) was allowed to percolate under gravity through stem segments in order to try to distinguish between these parts of the absorption process.

In *figs. 1* and *2* data are presented of two typical experiments with L-α-alanine. In explaining the curves obtained by plotting the amount of recovered C^{14} in each drop against the perfusion time or the percolated volume, a number of stages can be distinguished:

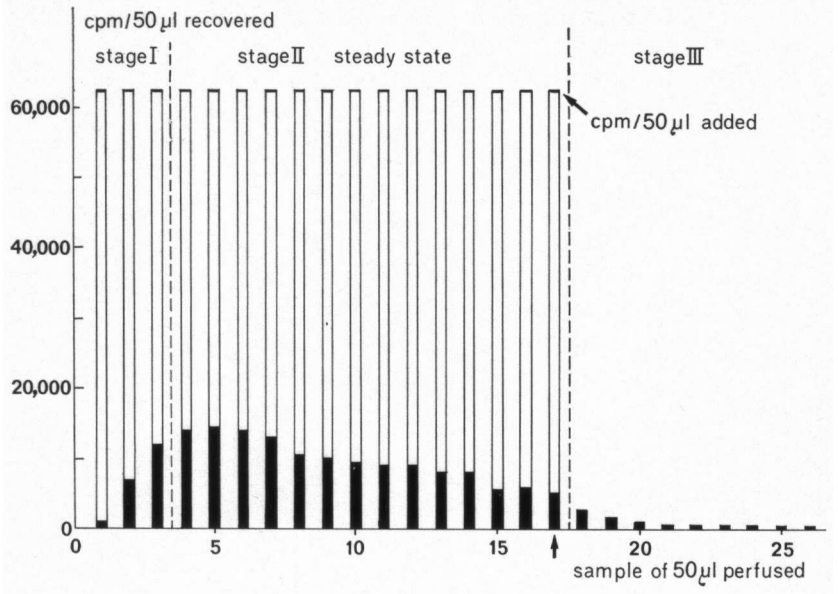

Fig. 1. The absorption of 0.005 M L-α-alanine during its flow through an isolated tomato stem segment of 148 mm. Total recovered ^{14}C-alanine: 15.2%. Average steady state delivery: 18.9%. Rate of flow 545 mm^{3}hr^{-1}. Number of xylem vessels 248. ↑ addition of 0.5 ml of distilled water. For explanation of the stages I, II and III see text.

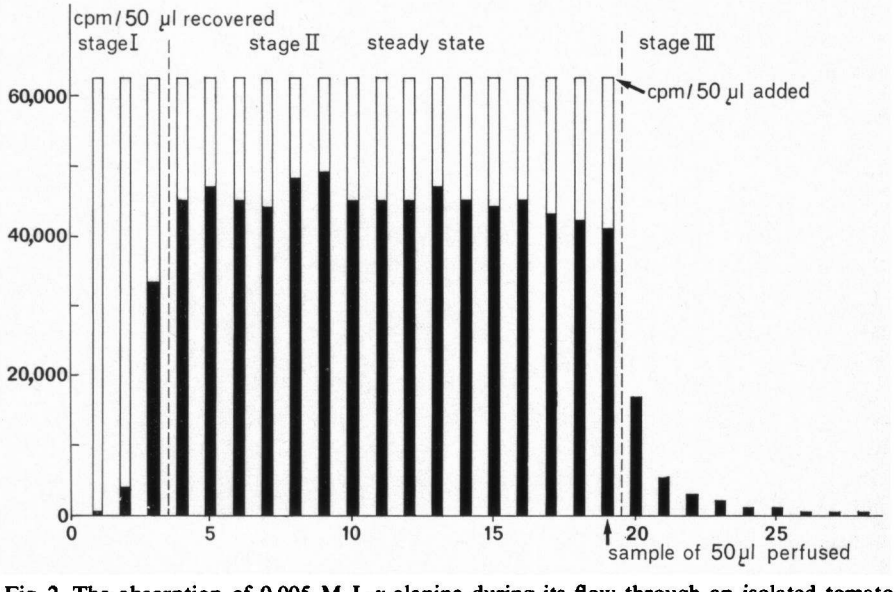

Fig. 2. The absorption of 0.005 M L-α-alanine during its flow through an isolated tomato stem segment of 132 mm length. Totally recovered ^{14}C-alanine 64.9%. Average steady-state delivery 74.6%. Rate of flow 417 mm^{3}hr^{-1}. Number of xylem vessels 85. ↑ addition of 0.5 ml of distilled water.

STAGE I represents the filling of the xylem vessels and vessel walls, the free space of the system.
(a) The first droplet recovered will be water present in the vessels and in the interstices of the vessel walls at the start of the experiment. A gradual replacement of these water molecules by the amino acid solution occurs.
(b) The first ^{14}C-amino acid molecules have passed the stem segment and are recovered. Their radioactivity level (cpm/ml), as a percentage of that of the supplied solution is low owing to the dilution with original vessel water.
(c) The ^{14}C-recovery level steadily increases and would reach the 100% level at an extrapolated time t_f if the filling of the free space were the only process involved.

STAGE II represents the steady-state delivery (absorption) level. Before the 100% level is reached, the ^{14}C-recovery level becomes approximately constant and remains so over a considerable part of the time of the experiment till all the supplied amino acid has entered the stem segment. In this stage a constant fraction of the amino acid molecules escapes from the passing solution. The slight decline could point to a gradual filling of vessels with small diameters.

STAGE III represents the washing-out of the amino acid from the free space. The activity recovered in this sharply declining part of the curve consists of ^{14}C present in the vessels and vessel walls – as far as these are freely accessible to the moving washing solution – diminished with the amounts which irreversibly escape during the washing period.
Fig. 2 demonstrates these three stages most clearly.

Based on a total recovery of 15,2% of ^{14}C-alanine in the experiment presented in *fig. 1* $*K_t$ of 0.0127 mm^{-1} can be calculated. Based on the steady-state delivery of 18.9% $*K_p = 0.0113\ mm^{-1}$.

The *K-value can also be calculated by direct analysis of the stem segment after its sectioning in a number of 10 mm parts. *Fig. 3* demonstrates the results obtained with the stem segment used in the experiment of *fig. 1*. The *K-value found by this procedure is $*K_s = 0.0123\ mm^{-1}$.

The *K-values computed in these three different ways, viz. 0.0127–0.0113–0.0123 mm^{-1}, lie closely together. With the obtained straight line in *fig.3* they seem to justify the approximations applied in this work.

In a second experiment (*fig. 2*) the *K-values are much lower: $*K_t = 0.0027$, $*K_p = 0.0022$. As the flow rates diverge little, it seems very likely to suppose the difference to lie in the number of xylem vessels, thus a larger absorbing surface might play an important role.

In another stem segment (length 114 mm, flow rate 625 mm^3hr^{-1}, number of xylem vessels 94) a perfusion experiment with L-α-amino-iso-butyric acid was performed. A similar absorption curve like those in *figs. 1* and *2* is obtained. The steady state delivery (96,9%), however, is higher than for alanine. From this delivery $*K_p = 0{,}0003\ mm^{-1}$ has been computed, which means $*K_p$-L-α-alanine is about 8 × $*K_p$-L-α-amino-iso-butyric acid (compare with *fig. 2*).

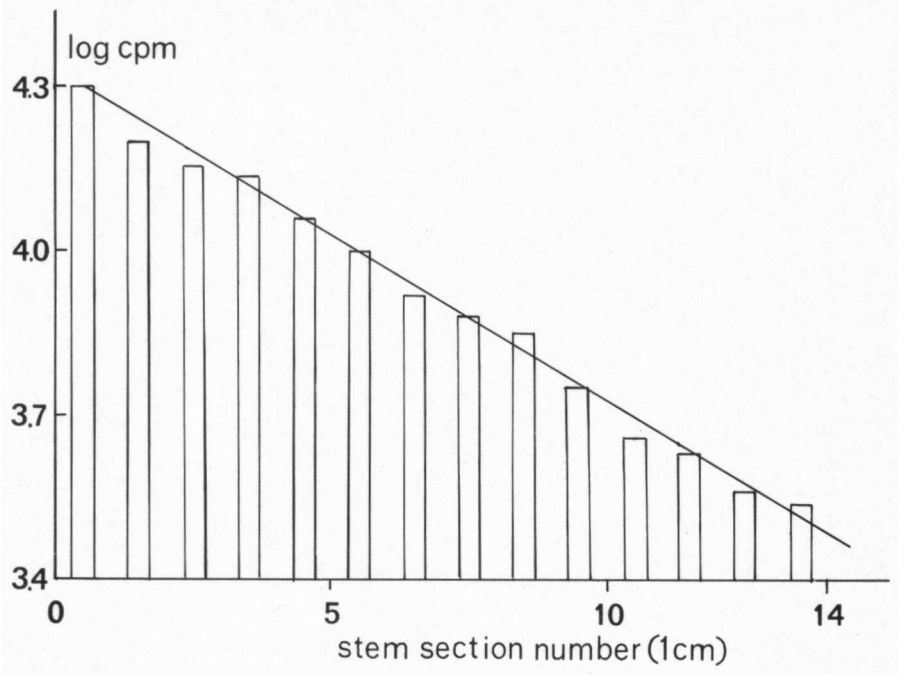

Fig. 3. Longitudinal activity distribution in the stem segment of 148 mm (see *fig. 1*) measured after its sectioning into pieces of 10 mm. Slope of the line characteristic for this very stem $^*K_s = 0.0123\ mm^{-1}$.

4.2. The influence of transpiration

In order to estimate the influence of transpiration on the escape of amino acids from the xylem vessels a stem segment was only partly covered with a plastic wrapping. A distinct difference in accumulation between the covered and the non-covered parts of the stem showed up (*fig. 4*). In the covered part $^*K_s$ is approximately 0.0038 mm^{-1}. In the uncovered part of the stem a new accumulation gradient seems to establish. Here $^*K_s = 0.0173\ mm^{-1}$ (approximately). Consequently, under conditions of higher or lower transpiration, the $^*K_s$ is about 5 times higher than in non-transpiring stems. This suggests that in the intact plant the stem will be a hardly invincible barrier for any L-α-alanine that would possibly move through the xylem in an upward direction.

4.3. The influence of the flow rate

Fig. 5 shows the influence of the flow rate itself on absorption from the vessel fluid. Using one stem segment the rate of flow was changed during the experiment by inclining the stem axis. This procedure yields two "steady-state" levels. The inverse relation between flow rate and $^*K_p$-value is clearly demonstrated.

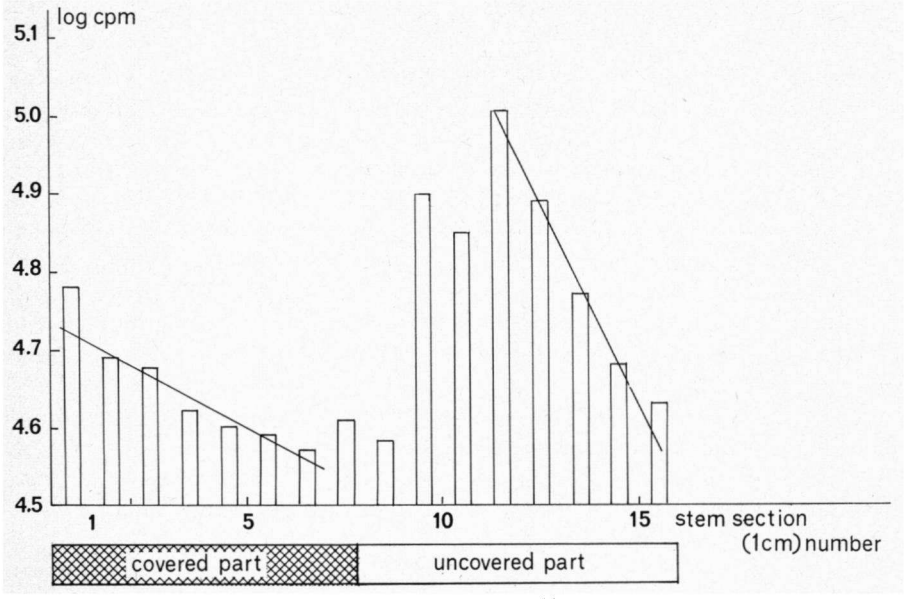

Fig. 4. Influence of the transpiration on the uptake of ^{14}C-alanine from a 0.005 M solution percolating through the xylem vessels. 7.8 cm of the stem has been wrapped in a plastic sheet; the remainder was uncovered and transpired normally. Length of the stem segment 158 mm. Rate of flow 857 mm^3hr^{-1}.

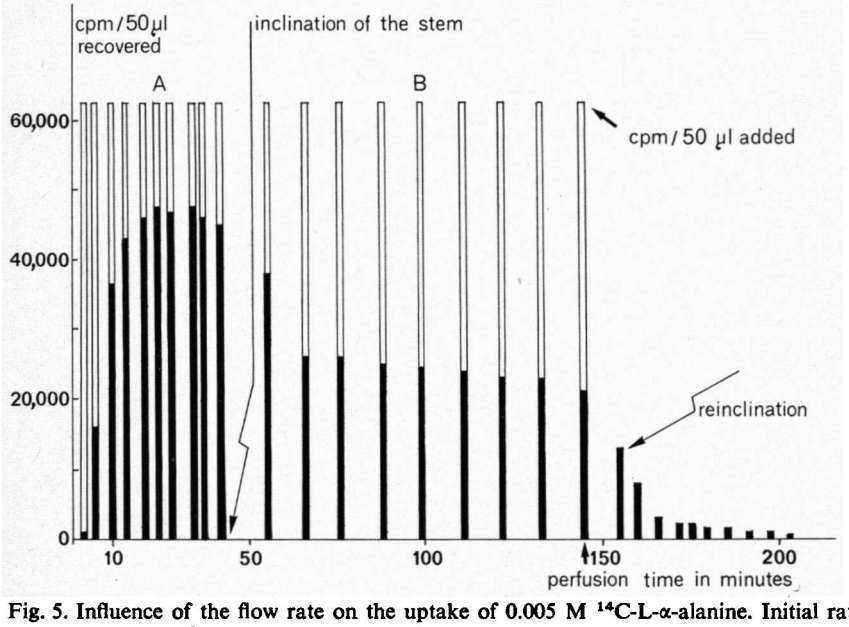

Fig. 5. Influence of the flow rate on the uptake of 0.005 M ^{14}C-L-α-alanine. Initial rate of flow (A) = 714 mm^3hr^{-1}, $*K_p$ (A) = 0.0025 mm^{-1}. After reducing the flow rate to 263 mm^3 hr^{-1} by inclining the axis of the stem over an angle of 60°, $*K_p$ (B) = 0.0073 mm^{-1}. ↑ addition of 0.5 ml of distilled water.

5. DISCUSSION

The model of an irreversible absorption during mass flow (HORWITZ 1958) offers an adequate basis for explaining the present results. In such a concept the rate of flow (A_pV) is very important for the absorption capacity. The flow rate depends on the number of xylem vessels and their volume (consequently their longitudinal absorption surface determines the capacity of the FS).

In his experiments with tomato plants DIMOND (1966) found that all xylem vessels were functional in translocation, but according to Poiseuilles law fluids are transported much faster through larger vessels than through smaller ones. He even computed that the single largest vessel of a population of 650 xylem vessels would conduct 23% of the water transport by the entire bundle per unit of time. Small differences in the number of large vessels therefore will greatly influence the flow rate. Consequently the number of xylem vessels is not simply directly related to the FS-capacity. If the volume of the xylem vessels rises, the exchange surface does not increase proportionally. In a later paper we hope to deal more extensively with this problem in connection with the mass flow character of the transport.

Since it seems highly improbable that the FS is less accessible for L-α-amino-iso-butyric acid than for L-α-alanine, the differences between their *K-values seem to be caused by different lateral transport rates of both substances. This could be the result of the expected differences in rate of metabolic conversion in the xylem surrounding cells: L-α-amino-iso-butyric acid is not metabolized.

The data are consistent with a two-phase model for the absorption of amino acids from xylem vessels:

A. a longitudinal flow through the vessels.

B. a lateral movement of the solutes from the vessel lumen into the walls and other free spaces and subsequently into the metabolic spaces of surrounding cells. Three stages may be distinguished:

(1) an initial absorption or exchange of charged molecules.

(2) free space diffusion through microcapillaries in the cell walls (STRUGGER and PEVELING 1961, TYREE 1969), which is essentially reversible.

(3) uptake into the metabolic spaces of surrounding cells.

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