Plant Physiol. (1979) 63, 244–247
0032-0889/79/63/0244/04/$00.50/0

Kinetics of L-Alanine Escape from Xylem Vessels

Received for publication April 10, 1978 and in revised form September 7, 1978

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ABSTRACT

Labeled (^3H or ^{14}C) L-alanine was perfused through the xylem vessels of isolated tomato internodes (*Lycopersicon esculentum* cv. Moneymaker) at various concentrations (10^{-6} molar to 10^{-2} molar). At each concentration the escape of L-alanine from the xylem vessels was apparently a first order process, which is in agreement with Horwitz' (1958, Plant Physiology 33:81-93) model for irreversible escape from the xylem vessels. The escape constant (K) decreased at higher concentrations of L-alanine, which implies that Horwitz' model is inappropriate to describe the kinetics of L-alanine escape, and that the escape at least partly is a saturable process. To obtain data that relate the concentration of L-alanine in the xylem vessels and the escape rate of the amino acid, average escape rates per internode were measured and the corresponding concentrations were calculated from the integrated form of the Michaelis-Menten equation.

As the concentration dependence of the escape rate was biphasic, three possible mechanisms were considered, escape being caused by: (*a*) saturable amino acid uptake of cells around the xylem vessels and diffusion into the free space; (*b*) saturable uptake of the cells around the xylem vessels, but at higher amino acid concentrations in the xylem vessels the number of cells, that participate in the uptake, increases; (*c*) two, simultaneously operating, saturable uptake systems in the cells around the xylem vessels.

In the root system of many higher plants inorganic nitrogen taken up from the soil is converted into organic nitrogen, part of which is translocated to the shoot via the xylem vessels. The main organic nitrogen compounds in the xylem sap are amino acids and amides (1, 13, 15). In tomato, the concentration of amino acids in the xylem bleeding sap is between 1 and 5 mM (8, 20).

Evidence has been obtained that under our experimental conditions, the free space of the xylem translocation path extends into the phloem area (18), so that direct transfer of amino acids from xylem to phloem may occur (*cf.* 9). The escape of amino acids from the transpiration stream is therefore, an important process in the nitrogen supply of stem and leaves. Investigations on this process in isolated internodes of tomato showed that in perfusion experiments the amount of amino acid that escapes from the xylem vessels is quantitatively absorbed by the surrounding living cells (16, 19, 21).

The kinetic data of the escape process have been interpreted, so far, by use of the model of Horwitz (5, 16, 17), in which the escape is regarded as a first order process. Inasmuch as amino acids are selectively absorbed from xylem vessels (16, 19, 21), it may be expected that the escape will show saturation kinetics. Such kinetic data have been also found for the uptake of amino acids by other plant tissues (2, 7, 11, 12, 22). The experiments described in this paper were carried out to obtain more information on the escape kinetics of amino acids from xylem vessels.

MATERIALS AND METHODS

Cultivation of Plants and Perfusion Technique. The cultivation of the plants (*Lycopersicon esculentum* cv. Moneymaker) and the perfusion technique have been described before (17). Briefly, labeled amino acids were perfused through excised tomato internodes and the radioactivity in the perfusate was counted by liquid scintillation spectrometry. All perfusion experiments were carried out at 27 C.

Perfusion Experiment. To determine the distribution of L-alanine that accumulated during a perfusion experiment along an internode, ^{14}C-labeled L-alanine and a different concentration of ^3H-labeled L-alanine were successively perfused. The labeled materials were removed from the xylem vessels by washing with distilled H_2O. After perfusion the internode was immediately cut into sections of 1 cm. Each section was ground in 0.2 ml of 70% (v/v) ethanol. A sample of 0.05 ml of the ethanolic extract was counted in 10 ml of scintillate (17) by liquid scintillation spectrometry. Quench corrections were made by a calculator program.

The concentration dependence of the escape rate of L-alanine was determined by perfusing internodes with ^{14}C-labeled L-alanine, and measuring the escape rate during the steady-state (16, 17). Each internode was perfused by three to five different concentrations of L-alanine, the lowest concentration first; for each internode a different set of concentrations was used. To be sure that L-alanine escape was similar in the various internodes, the escape at one concentration was determined in at least two internodes. All of the ^{14}C-labeled material leaking out was alanine as determined by paper chromatography (*cf.* 21).

Fitting Procedure. Data relating escape rate and concentration were fitted to the equations 6 and 7 by means of an iterative computer program for nonlinear least squares regression.

Radioactive Materials. L-[U-^{14}C]Alanine (10 mCi/mmol) and L-[2,3-^3H]alanine (1 Ci/mmol) were supplied by the Radiochemical Centre, Amersham, UK.

RESULTS

In earlier reports on the escape of sugars and amino acids from xylem vessels (16, 17) Horwitz' model (5) for irreversible escape from porous pipes was used. In this model (5) the escape is considered to be a first order process. This process, therefore, can be characterized by a single parameter, the escape rate constant K.[1]

If the escape is a first order process, the amount of solute which as accumulated in the internode by irreversible escape from the

[1] Abbreviations: A: amount of amino acid absorbed per internode (moles/internode); C_o: dpm per 0.05 ml in the solution applied at the top of an internode; C_p; dpm per 0.05 ml in the solution collected at the lower end of an internode during the steady-state uptake (16, 17); A_p, : transverse-sectional area of the xylem vessels (mm^2); V: linear velocity of flow (mm hr^{-1}); A_pV: mass flow rate of the perfusing fluid (mm^3 hr^{-1}); L: length of an internode (mm); K: escape constant (mm^2 hr^{-1}); F: total volume of the xylem vessels in an internode (mm^3); $\bar{v}$: average escape in an internode (moles mm^{-1} hr^{-1}); S_o: amino acid concentration in the solution supplied to the top of an internode (molar); S_p: amino acid concentration in the perfusate at the lower end of an internode during steady state escape (molar); S_x: concentration of amino acid at which the escape rate equals $\bar{v}$ (molar).

xylem vessels will decrease exponentially from the top to the lower end. This was actually found, when ^{14}C-labeled or ^{3}H-labeled L-alanine was perfused through tomato internodes (Fig. 1). Plotting the logarithm of the amount of ^{14}C or ^{3}H in 1 cm of internode against the distance from the top results in a straight line, the slope of which is equal to $-K$.

Alternatively, the value of K can be calculated from the relative amount of radioactive L-alanine recovered in the perfusate at the lower end of the internode by use of equation 1 (5, 16):

$$K = (A_pV/L).\ln (C_o/C_p) \quad (1)$$

Table I shows that both methods yield approximately the same K-values.

Although for each individual experiment the escape seems to be a first order process, Figure 1 clearly shows that lower K-values were obtained when higher concentrations of L-alanine were perfused. This result may indicate that the escape of L-alanine is a saturable process.

As there is no linear relation between the escape rate and the concentration of L-alanine in the vessels, we measured the average escape rate in the internode ($\bar{v}$) and calculated the corresponding concentration (S_x) as follows. The amount of amino acid absorbed by the internode, when a volume F of amino acid solution has perfused, is:

$$A = F.S_o(C_o - C_p)/C_o \quad (2)$$

As the perfusion rate is equal to A_pV, the time (t) during which a volume F was perfused is equal to F/A_pV. The average escape rate per unit length of internode is:

$$\bar{v} = A/Lt = A_p.V.S_o(C_o - C_p)/(L.C_o) \quad (3)$$

If the escape follows Michaelis-Menten kinetics, the local concentration in the xylem vessels (S_x), where the escape rate equals the average escape rate ($\bar{v}$), follows from the integrated Michaelis-Menten equation:

$$S_x = (S_o - S_p)/\ln(S_o/S_p) \quad (4)$$

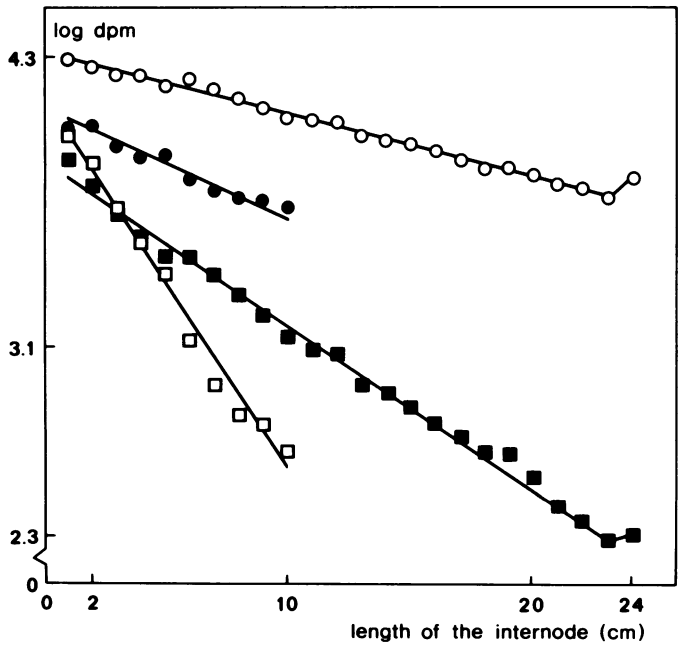

FIG. 1. Distribution profiles of radioactivity along two internodes. Internode 1 (length 100 mm; flow rate 744 mm^3 hr^{-1}) was successively perfused by 1 ml 5×10^{-4} M L-[^{14}C]alanine (□——□), 0.5 ml distilled H_2O, 1 ml 10^{-2} M L-[^{3}H]alanine (●——●), and 0.5 ml distilled H_2O. Internode 2 (length 240 mm; flow rate 678 mm^3 hr^{-1}) was successively perfused by 1 ml 5×10^{-4} M L-[^{14}C]alanine (■——■), 0.5 ml distilled H_2O, 1 ml 5×10^{-3} M L-[^{3}H]alanine (○——○), and 0.5 ml distilled H_2O.

Table I. Lateral escape constants (K) of alanine at different concentrations.
(a) Computed by equation 1 from the dpm that leaked out in the steady state.
(b) From the ^{14}C-content along the internode after perfusion (see Fig. 1) the lateral escape was computed from the slope of the line (-K).

	5×10^{-4} M		5×10^{-3} M		10^{-2} M	
	(a)	(b)	(a)	(b)	(a)	(b)
internode 1	16.2	18.4			5.3	4.7
internode 2	8.7	10.1	4.4	4.0		

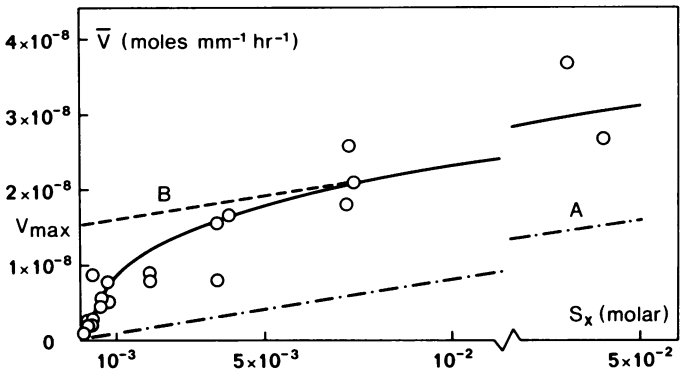

FIG. 2. Relation between concentration and escape rate of L-alanine from xylem vessels of tomato internodes. Average escape velocity in an internode ($\bar{v}$) was computed from equation 3 and the concentration S_x, at which the escape velocity is equal to $\bar{v}$, was calculated according to equation 5. Line A (-·-·-·-) indicates apparent linear component of escape; line B (-----) parallel to A yields V_{max}.

or, as $S_o/S_p = C_o/C_p$:

$$S_x = S_o((C_o - C_p)/C_o)/\ln(C_o/C_p) \quad (5)$$

The right-hand side of equation 5 is also a good approximate solution for S_x in case of two simultaneously operating Michaelis-Menten systems (Borstlap and Doucet, unpublished results).

The data for the concentration dependence of the L-alanine escape rate, obtained in this way, are shown in Figure 2. The escape rate levels off at about 2×10^{-3} M, but continues to increase up to a concentration of 5×10^{-2} M. A Hofstee plot of the same data (Fig. 3) shows that the kinetics deviates from the Michaelis-Menten type.

It is not clear whether the increase in the escape rate at higher concentrations is due to a linear component, or to a second saturable component.

First, we fitted the data to the equation:

$$\bar{v} = V_{max}.S_x/(K_m + S_x) + k.S_x \quad (6)$$

where V_{max} is the maximal uptake rate and K_m is the apparent Michaelis constant of the saturable component, and k is the rate constant of the linear component. The values of the kinetic parameters were: $K_m = 1.3 \times 10^{-3}$ M; $V_{max} = 1.5 \times 10^{-8}$ mol mm^{-1} internode hr^{-1}; $k = 9 \times 10^{-7}$ mol mm^{-1} internode hr^{-1} M^{-1}.

Second, the data were fitted to the equation for two saturable systems:

$$\bar{v} = V_{max1}.S_x/(K_{m1} + S_x) + V_{max2}.S_x/(K_{m2} + S_x) \quad (7)$$

where V_{max1} and V_{max2} are apparent maximal uptake rates, and K_{m1} and K_{m2} are apparent Michaelis constants of the high affinity and low affinity system, respectively. The parameter values for the high affinity system were $K_{m1} = 1.5 \times 10^{-4}$ M and $V_{max1} = 2.0 \times 10^{-9}$ mol mm^{-1} internode hr^{-1}; and for the low affinity system,

$K_{m2} = 9 \times 10^{-3}$ M and $V_{max2} = 5.2 \times 10^{-8}$ mol mm^{-1} internode hr^{-1}.

To compare the kinetic parameters with those obtained for L-alanine uptake in other plant materials, V_{max} values were also expressed per mg dry weight of tissue (Table II). From earlier data (18) it could be calculated that the dry weight of the cells involved in the amino acid uptake in the internodes amounts to 0.34 mg/mm of internode. The parameters for L-alanine escape from the xylem vessels appeared to be in good agreement with those for L-alanine uptake in other plant tissues (Table II).

DISCUSSION

The amino acid escape from the xylem vessels showed first order kinetics in each perfusion experiment, as indicated by a constant K-value along each internode (Fig. 1). The apparent rate constant of escape (K) decreased with increasing concentrations of perfusing amino acids, which suggests saturation kinetics of the escape process (Fig. 1). The explanation for these seemingly conflicting results is that the concentration range along the internode was either in the range of first order kinetics ($S_x \leq 5 \times 10^{-4}$ M), or in the range where the escape rate increases approximately linearly with the concentration ($S_x > 2 \times 10^{-3}$ M).

We have calculated the distribution of L-alanine along the internode after perfusion, using equations 6 and 7. The resulting lines for the escape of L-alanine along the internode were almost straight in semilogarithmic plots and hardly deviated from the lines shown in Figure 1.

The kinetics of the escape of alanine from the xylem vessels suggests different possibilities as to the mechanism involved. (*a*) The escape rate may be interpreted to be composed of a saturable and a linear component (equation 6). The saturable component may be ascribed to carrier-mediated uptake by the cells surrounding the xylem vessels, and the linear component may be due to diffusion into the apoplastic space. The presence of

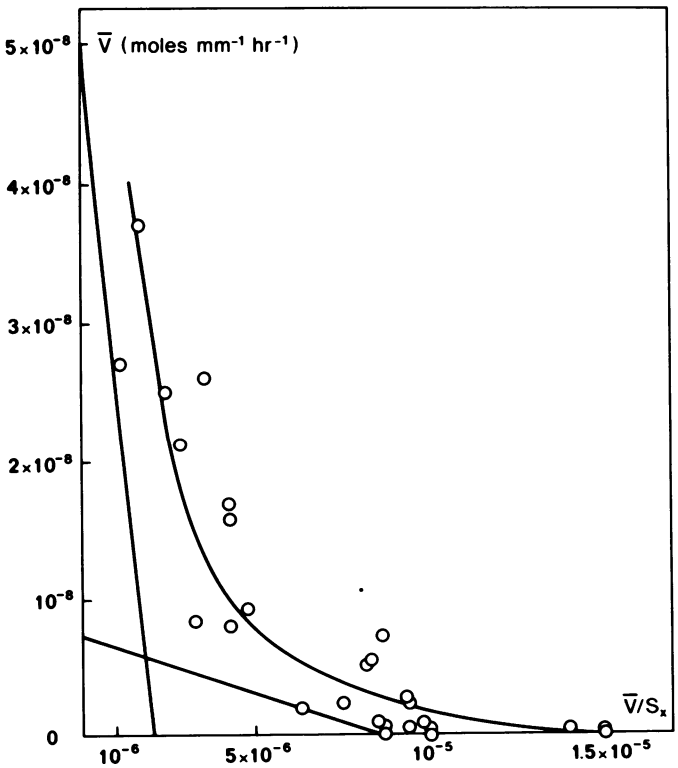

FIG. 3. Hofstee plot for the escape of L-alanine in internodes. Same data as in Figure 2 were plotted. Straight lines represent the two simultaneously operating transport systems.

Table II. The K_m- and V_{max}-values for L-alanine escape in tomato internodes in case of a dual uptake mechanism (Fig. 3) in comparison with the L-alanine uptake in some other plant tissues. The data were adopted or computed from (2, 7). The K_m and V_{max} are expressed, respectively, in M and in nmoles mg^{-1} dr. wt. h^{-1}.

	K_{m1}	K_{m2}	V_{max1}	V_{max2}
tomato internode	1.5×10^{-4}	9.0×10^{-3}	6.0	155
Spirodela polyrhiza	3.5×10^{-5}	1.5×10^{-3}	7.8	30
Soya callus	4.6×10^{-5}	8.0×10^{-3}	3.1	97

a linear component in initial uptake rates has been observed in experiments with plant tissues and has been attributed to filling of the free space by the solute (7, 11, 22). In our experiments with internodes, filling of the free space with solutes lasts not more than 10 to 15 min (18). The escape rate of 5 mM L-alanine was, however, constant for at least 6 hr (Van Bel, unpublished results). This implies that filling of the free space in itself did not contribute substantially to the escape rate. (*b*) A net escape of amino acid from the xylem vessels by diffusion into the apoplastic spaces of the internodes may be maintained when the amino acid is taken up by the cells located along the diffusion path. The cellular uptake will result in a concentration gradient of L-alanine, extending from the xylem vessels into the apoplastic spaces of the surrounding tissue. Because of the saturation kinetics this gradient will be very steep at low amino acid concentrations in the xylem vessels, but will be more flat at higher concentrations. As a consequence, amino acid uptake will be confined to a small number of cells around the xylem vessels at low concentrations, whereas a larger number of cells will participate in uptake at higher concentrations. The same reasoning has been given by Ehwald *et al.* (3) to explain the biphasic kinetics of sugar uptake in maize root tips. (*c*) The kinetics may be interpreted as due to two saturable components (equation 7). This interpretation, in which uptake is thought to be due to the activity of two independent transport systems, has been given for the uptake of α-aminoisobutyric acid by barley leaf tissue (12), and for the uptake of several neutral amino acids by intact fronds of duckweed (2).

We prefer to interpret the kinetics of L-alanine escape according to the model advanced by Ehwald *et al.* (3). First, it is conceptually obvious that amino acids escaping from the xylem vessels enter the apoplastic spaces of the surrounding tissues before cellular uptake can occur. Second, the volume of the free space of the xylem translocation pathway has been estimated by experiment, and amounts to 2.5 times the volume of the xylem vessels (18). We realize that in some cases the biphasic kinetics in amino acid uptake by plant tissues cannot be explained by the model of Ehwald *et al.*, as such kinetic behavior has been found also in cell suspensions (4, 7).

Can our results contribute to knowledge on the translocation and mass transfer of amino acids in the sieve tubes? Direct measurements of the escape rates of amino acids from the sieve tubes have not been reported. Yet, longitudinal mass transfer rates in the phloem have been measured (6, 10, 14) and they can serve as a measure for the escape, as the latter is inversely proportional to the mass transfer rate (16, 17). Different escape rates for amino acids occurred in soybean (14), although other experiments did not confirm these findings (6). We cannot say from the present information whether the kinetics for amino acid escape from the sieve tubes resembles those for the escape from the xylem vessels. It is not clear whether the role of the free space is as important as in the xylem vessel escape.

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