

Evidence for Distinct Amino Acid Transport Systems in Cultured Tobacco Cells

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It is shown by competition experiments that tobacco cell lines have distinct transport systems for L-amino acids. For all tested amino acids the Lineweaver-Burk plots were diphasic indicating the presence of more than one carrier for any one amino acid. Moreover distinct transport systems for neutral, acidic and basic amino acids were kinetically characterized. Based on competition experiments neutral amino acids were absorbed by all transport systems. Aspartic acid entered the cells via its own carrier and via the basic carrier while arginine was taken up only by the basic carrier. Neutral amino acids such as L-leucine or L-phenylalanine were taken up faster than acidic or basic amino acids.

While characterizing a *p*-fluorophenylalanine (PFP)-resistant line of tobacco cell cultures (*Nicotiana tabacum* L. cv. Xanthi) it was shown that a generally reduced uptake of all amino acids might cause the resistance of this line [1]. The K_m -values for L-phenylalanine uptake, however, were identical in the sensitive (TX1) and the resistant (TX4) cell line. Several neutral L-amino acids inhibited the uptake of L-phenylalanine while aspartic acid or arginine did not reduce the uptake of L-phenylalanine [1]. This indicated the presence of distinct amino acid transport systems. Since the uptake of all amino acids was greatly impaired, it was concluded that all amino acid carriers of tobacco cells share a common component or, that an inhibitor for all amino acid carriers caused the general effect.

Now we present a more detailed kinetic study of the uptake of various amino acids which clearly confirms the presence of different amino acid transport systems in tobacco tissue cultures.

Materials and Methods

Plant Material: Growth conditions for the PFP-sensitive and the PFP-resistant cell line have been described previously [1, 2]. TX1 is the sensitive and TX4 is the resistant cell line.

Uptake Experiments: Cells in logarithmic growth phase with a density of 0.8 g fresh weight per 10 ml medium were used throughout in all uptake experiments. For a typical experiment several flasks of 70 ml were combined and shaken uncovered for

4 hours. Five ml of this suspension were incubated with the appropriate labelled amino acid and the inhibitory compound during competition experiments. The cells were collected after 10 minutes in the case of TX1 cells and after 30 minutes in the case of TX4 and extracted as described previously [1]. To distinguish between carrier-mediated uptake and diffusion, cells were incubated under identical conditions in the presence of 1 mM 2,4-dinitrophenol which completely inhibits carrier-mediated uptake. The uptake rate was then calculated from the difference between the incubations with and without dinitrophenol.

Results

TX1 cells absorbed various L-amino acids with different velocities. The neutral amino acids L-leucine and L-phenylalanine were taken up more quickly than acidic and basic amino acids (Fig. 1). Tryptophan uptake was comparatively slow. As shown in Fig. 1, that pattern of uptake velocities for the amino acids was also found for TX4 cells (shaded blocks). However, the total rate of uptake by TX4 was nearly 6–8 times slower than by TX1. The fact that the cell lines only differ in uptake velocities further indicate that the transport systems for the various amino acids are identical in both cell lines. For an unknown reason, however, the transport systems of TX4 do not reach the uptake velocities of TX1.

Uptake of L-arginine

A Lineweaver-Burk plot for L-arginine showed a diphasic pattern indicating the presence of more than one carrier system for the uptake of L-arginine

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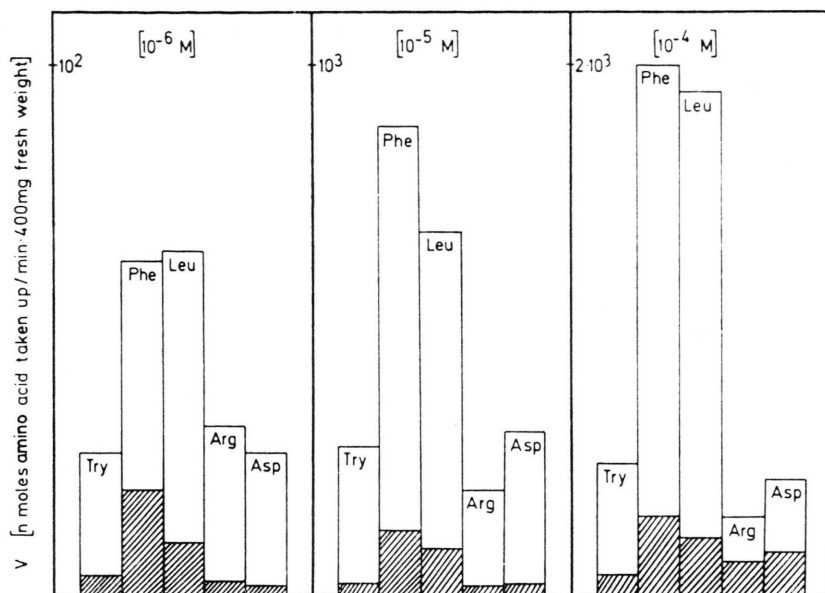


Fig. 1. Comparison of the uptake velocities for various ^{14}C -labelled L-amino acids (tryptophan, leucine, arginine, aspartic acid, phenylalanine) at different amino acid concentrations by TX1 and TX4 cells (shaded blocks).

(Fig. 2). Diphasic patterns were found for all other tested amino acids (L-phenylalanine, L-leucine, L-tryptophan and L-aspartic acid) by TX1 and TX4 cells. The system with the higher K_m was never characterized during this study. The K_m -value for arginine was $1.2 \times 10^{-5}\text{ M}$ (Fig. 2) for the more specific system [3] of TX1 cells. For TX4 cells, a value of $7 \times 10^{-5}\text{ M}$ was measured. Such a big difference in K_m -values of one amino acid was only found with L-arginine. Competition ex-

periments were carried out with several L-amino acids. The uptake of L-arginine was competitively inhibited by the other basic amino acid, L-lysine (Fig. 2). Neutral L-amino acids such as L-alanine (Fig. 2), L-leucine and L-phenylalanine (not shown) were also very good competitive inhibitors. Aspartic acid reduced the uptake of L-arginine to a lesser degree (Fig. 2). However, L-arginine did not inhibit the uptake of L-aspartic acid (Fig. 3) or any neutral L-amino acid significantly.

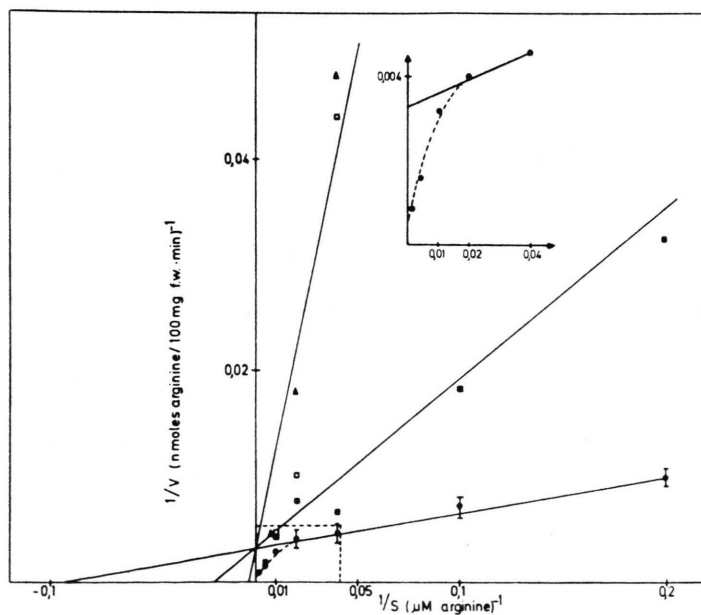


Fig. 2. Lineweaver-Burk plots of L-[U- ^{14}C]arginine uptake by TX1 cells without (●-●) and with 250 μM L-aspartic acid (■-■), L-lysine (△-△) and L-alanine (□-□).

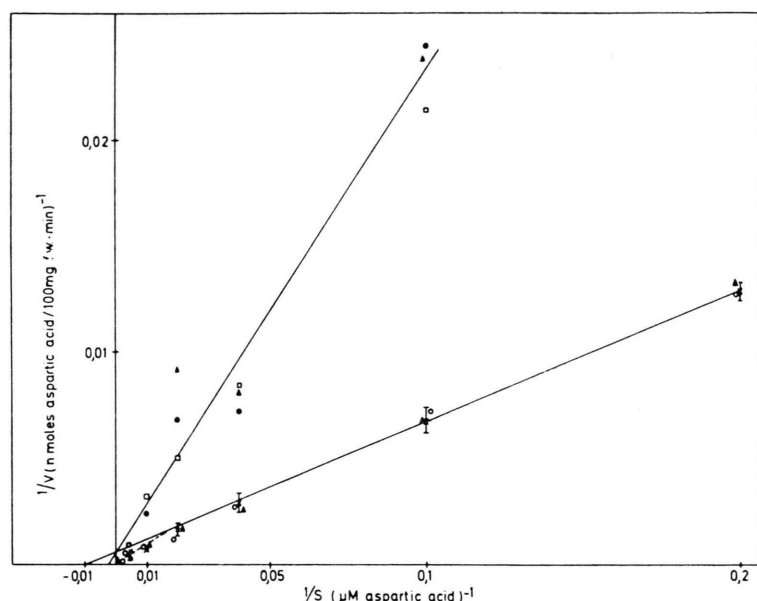


Fig. 3. Lineweaver-Burk plots of L-[U-¹⁴C]-aspartic acid uptake by TX1 cells without (×-×) and with 250 μM L-glutamic acid (○-○), L-arginine (△-△), L-alanine (●-●), L-asparagine (▲-▲) and L-phenylalanine (□-□).

Uptake of L-aspartic acid

The K_m -value for L-aspartic acid was 10^{-4} M in TX1 (Fig. 3) and TX4. Competition experiments were carried out with neutral, acidic and basic L-amino acids. It was surprising that L-glutamic acid effected the uptake of L-aspartic acid only very slightly (Fig. 3) while several neutral L-amino acids were strong inhibitors of the aspartic acid uptake system. It has to be noted that L-aspartic acid did not inhibit L-phenylalanine uptake [1] nor the uptake of other neutral L-amino acids.

Uptake of L-leucine

For L-leucine, K_m -values of 3.5×10^{-5} M and 5×10^{-5} M were measured for TX1 and TX4, respectively. L-alanine and L-phenylalanine competitively inhibited the leucine uptake while L-arginine and L-aspartic acid did not interfere with the leucine uptake. Some other amino acids, *e. g.* L-methionine, also reduced the rate of leucine uptake. The effect of increasing concentrations of alanine, phenylalanine and arginine (Fig. 4) on leucine uptake (10^{-4} M) clearly supports the findings for competition in Lineweaver-Burk plots.

Uptake of L-tryptophan

For the tryptophan uptake system, K_m -values of 0.9×10^{-4} M and 10^{-4} M were calculated for

TX1 and TX4, respectively. Leucine, alanine and phenylalanine inhibited greatly the uptake of tryptophan (Table I) while aspartic acid and arginine

Table I. Uptake of L-tryptophan at various concentrations in the presence of an inhibitory L-amino acid (2.5×10^{-4} M).

Inhibitor (2.5×10^{-4} M)	nmol L-tryptophan taken up at concentrations of					
	5 μM	10 μM	25 μM	50 μM	100 μM	500 μM
None	65	101	219	458	707	2478
Leucine	2	17	30	56	346	526
Alanine	0	0	0	0	52	452
Phenylalanine	2	3	15	24	231	552
Arginine	61	91	161	372	610	2347
Aspartic Acid	52	83	150	425	698	2360
Lysine	—	—	—	—	597	—
Methionine	—	—	—	—	69	—
Histidine	—	—	—	—	399	—
Valine	—	—	—	—	171	—

or lysine reduced the uptake only to a smaller extent. The effect of increasing concentrations of phenylalanine and arginine on the tryptophan uptake (10^{-4} M) is shown in Fig. 5. Even high concentrations of arginine did not reduce tryptophan uptake while the inhibition by phenylalanine was dependent on the concentration used. Since tryptophan and arginine were absorbed with comparable velocities by TX1 cells (Fig. 1) it can be concluded

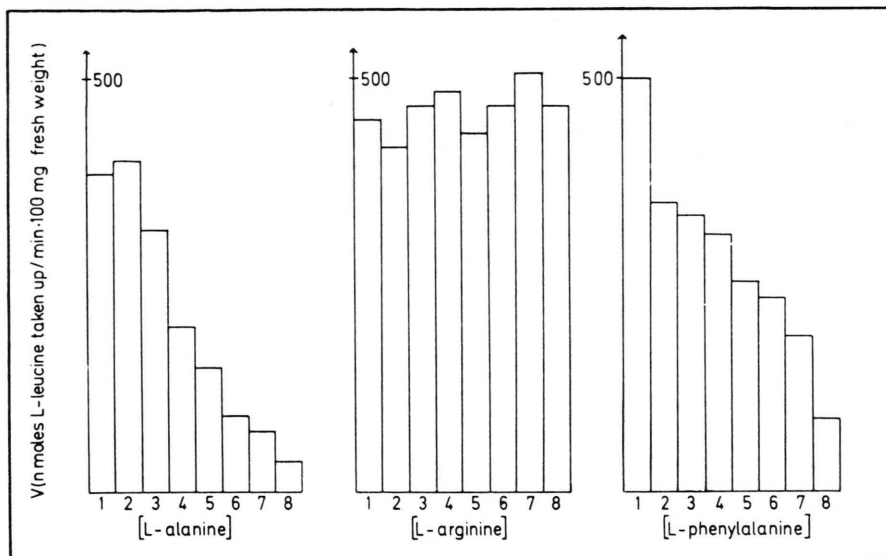


Fig. 4. Uptake of 10^{-4} M L-[U- 14 C]leucine in the presence of increasing concentrations of L-phenylalanine, L-alanine and L-arginine. Concentrations 1–8 denote 5, 10, 25, 50, 100, 250, 500 and 1000 μ M. Control: 491 nmol/min \pm 9.5%.

that the inhibition pattern found for the various amino acids were not due to the different uptake velocities.

Discussion

All amino acids tested were evidently transported into the cells of the tobacco lines by carriers. The Lineweaver-Burk plots indicated in all cases the presence of more than one transport system for any single amino acid. The system or the systems

with the higher K_m were not characterized since such carriers are not likely to be important for the cells. They would be only operative at high concentrations of amino acids not found under normal physiological conditions.

From the competition experiments one has to postulate the existence of at least one neutral, one basic and one acidic amino acid carrier. The tested neutral amino acids phenylalanine [1], leucine and tryptophan as well as several others (alanine, methionine, valine) were transported by all

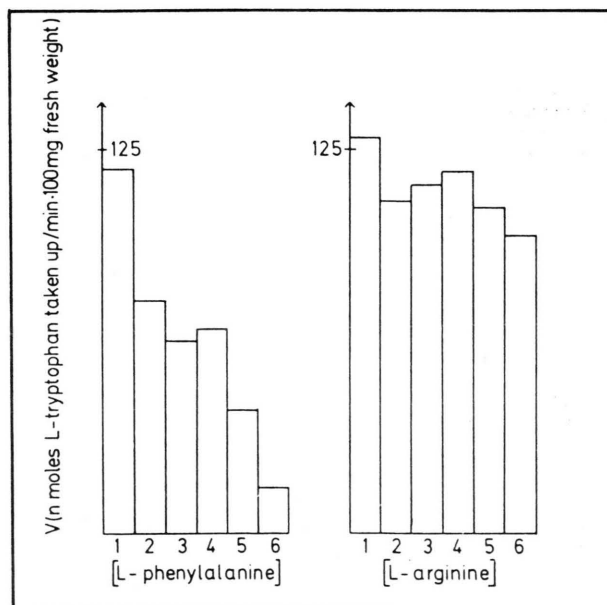


Fig. 5. Uptake of 10^{-4} M L-[U- 14 C]tryptophan in the presence of increasing concentrations of L-phenylalanine and L-arginine. Concentrations 1–6 denote 5, 10, 25, 50, 100 and 500 μ M. Control: 130 nmol/min \pm 16.2%.

carrier systems since they had a pronounced effect on the uptake of aspartic acid and arginine. It has been reported that in soybean cell cultures methionine, tryptophan, leucine and phenylalanine inhibited the uptake of glutamate, alanine and arginine [4]. This is in agreement with our findings. Arginine and aspartic acid, however, cannot enter the cells via the neutral amino acid carrier. Since the binding affinities for these amino acids were comparable to those found for neutral amino acids, they have to be transported by different carrier systems.

It was not intended by this study to distinguish between different carrier systems for one group of amino acids. Thus, it cannot be decided yet whether all neutral amino acids are transported by only one neutral carrier. Evidence for a very specific tryptophan carrier was presented by Widholm [5]. He selected 5-methyltryptophan resistant cells of carrots which became resistant due to a specific inhibition of tryptophan/5-methyltryptophan uptake. Leucine uptake was unchanged in wild type and resistant cells [5].

A specific carrier for basic amino acids became most evident, when we found that lysine could only inhibit the arginine uptake. Specific carriers for lysine and arginine have been postulated for soybean cultures [4] and sugarcane cultures [6]. The basic carrier can be used by a variety of neutral L-amino acids and even aspartic acid is transported to some degree by this carrier. Such overlapping of transport systems complicates the kinetic interpretation since more than one system contributes to the uptake.

Aspartic acid should be transported by an acidic transport system. This carrier was also used by

several neutral amino acids. In soybean cells [4] glutamate uptake was not inhibited by aspartic acid. In tobacco cells the uptake of L-aspartic acid was not inhibitable by glutamic acid. This unexpected result cannot be explained by a very slow absorption of aspartic acid by tobacco cells as was explained for soybean cells [4]. This result may even indicate the existence of two acidic carrier systems. On the whole, the specificities found for tobacco cells agree very well with those found in soybean cells [4].

The present study was undertaken to prove the presence of distinct amino acid transport systems. The uptake of all amino acids was found to be slower in TX4 cells compared to the wild type TX1. Our results clearly rule out that this effect is due to an impaired common amino acid carrier in TX4 cells. It is more likely that a pleiotropic mutation causes this generally reduced uptake of amino acids [1]. The overlapping of distinct carrier systems in their substrate specificities makes this even more likely. Pleiotropic effects in the amino acid transport of higher plants have not been reported previously due to the lack of proper mutants. With the possibility of selecting uptake mutants in cultured cells of higher plants, common components of distinct amino acid transport system may be detected as in fungal and bacterial systems [7–9]. However, it cannot be excluded at the moment that a common inhibitor for the uptake of L-amino acids is responsible for the reduced uptake of amino acids by TX4 cells [1].

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