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LIGHT-STIMULATED BIPHASIC AMINO ACID UPTAKE BY XYLEM PARENCHYMA CELLS

A.J.E. VAN BEL and C. VAN DER SCHOOT

Botanical Laboratory, State University of Utrecht, Lange Nieuwstraat 106, 3512 PN Utrecht (The Netherlands)

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SUMMARY

The secondary xylem of tomato (*Lycopersicon esculentum* cv Moneymaker) internodes was isolated and was shaken in solutions of ^{14}C-labelled amino acids.The uptake showed biphasic Michaelis-Menten kinetics. Light stimulated both uptake systems. During the uptake of amino acids the pH of the medium rose, more in the light than in the dark. Addition of 30 mmol l^{-1} K^+ to the medium retarded the pH rise. High K^+ concentrations (> 5 mmol l^{-1}) decreased the uptake, whereas low K^+ concentrations (< 2 mmol l^{-1}) stimulated it. The observations indicated amino acid/proton co-transport into xylem parenchyma cells.

INTRODUCTION

It is generally found that the uptake of amino acids by cells of higher plants obeys biphasic kinetics [1—6]. The biphasic nature has been explained in several ways:

(a) two independently operating carrier systems [4];
(b) one carrier system operating in the plasmalemma and a linear, probably diffusional component [6].

Additionally, two other explanations for biphasic kinetics have been given in papers concerning sugar uptake:

(c) one carrier system occurring in a protonated high-affinity form and in an unprotonated low-affinity form [7]; and
(d) especially for the uptake by multilayered tissues, one carrier system in

Address all correspondence to: Dr. A.J.E. van Bel, Botanisch Laboratorium, Lange Nieuwstraat 106, 3512 PN Utrecht, the Netherlands.

the plasmalemma. The distribution of substrate in the apoplastic space at different concentrations causes a biphasic isotherm [8].

In tomato internodes, the alanine escape from the xylem vessels appeared to be biphasic [9] and the escape of amino acids from the xylem vessels is accompanied with proton transport [10,11]. The escape might reflect the uptake activity of the phloem tissues, since it has been demonstrated that amino acids can rapidly traverse to the phloem [12]. But, more probably, the escape is due to the action of the paratracheal and the ray parenchyma cells, since these cells , which surround the tomato xylem vessels, closely connect with the vessels [13].

Therefore, we investigated whether the amino acid escape mechanisms, characterised by proton co-transport and biphasic kinetics, occur in xylem parenchyma cells.

MATERIAL AND METHODS

Tomato plants (*Lycopersicon esculentum* cv Moneymaker) were grown under conditions as described earlier [14].

Internodes with a well-developed xylem ring (2—3 mm thick) were excised and, after that a longitudinal incision was made, the outer soft layer (epidermis, cortex, phloem) of the stem was stripped off. The remaining cylinder was longitudinally cut into two parts and the medulla parenchyma and the internal phloem were removed with a scalpel. The strips of secondary xylem were cut into pieces (5 × 10 mm) and were shaken in distilled water (25°C) for 3 h under TL-illumination (17 W/m^2) or in the dark. Subsequently, the xylem pieces (150 mg fresh wt/treatment) were shaken in [^{14}C]amino acid solutions at 25°C in a water bath. The disappearance of ^{14}C from the medium was a measure for the rate of uptake. In light experiments the illumination was equal to that during the prewashing period.

The radioactive amino acids (spec. act. 10 mCi/mol) were purchased from the Radiochemical Centre, Amersham, UK.

RESULTS

In the following experiments it was attempted to influence some determinants of the proton-motive force. If the amino acid uptake is changed by these treatments, this is indicative for amino acid/proton co-transport. With reference to the chemiosmotic hypothesis, the proton-motive force is assumed to be determined by the ΔpH, the proton gradient across the plasmalemma, and ΔE, the membrane potential. In the present experiments the pH in the media was 6 (unless stated otherwise) and the ΔpH was therefore negligible.

Light is one of the means to excite proton extrusion [15,16]. The energy for the proton extrusion is thought to be supplied, at least partially, by the photosynthesis [15]. In xylem parenchyma cells, the use of light for this purpose promised to be successful, as microscopic observations showed

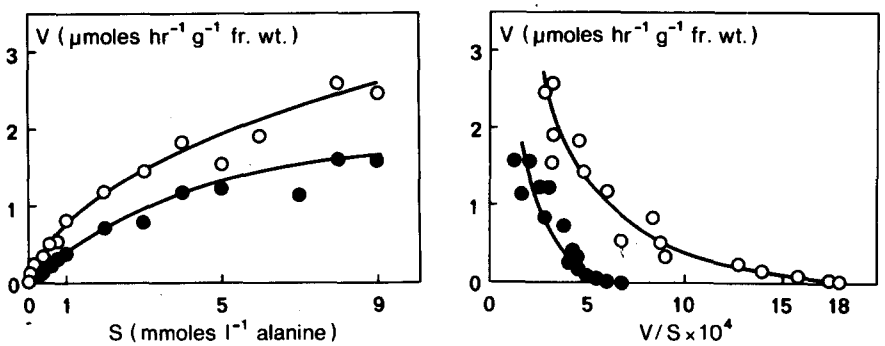

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Fig. 1. (a) Concentration-dependent uptake of alanine in the light (○——○) and in the dark (●——●). The xylem pieces were shaken for 6 h in alanine solutions in distilled water. (b) Hofsteeplot for alanine uptake in the light (○——○) and in the dark (●——●).

that the ray cells contain large chloroplasts. Light stimulated the uptake of alanine. The uptake showed biphasic kinetics (Fig. 1a) and light had a stimulating effect on both systems (Fig. 1b).

A signal for proton co-transport is that the pH of the medium rises more in the presence than in the absence of substrate [17]. The pH of the media with alanine rose more rapidly than in media without alanine both in the light and in the dark (Fig. 2). In the light the final pH was higher than in the dark (Fig. 2).

The uptake of substrates is inhibited by high concentrations of K^+ which affect the membrane potential [18,19]. With increasing concentrations of K^+ (> 5 mmol l^{-1}) the uptake of alanine and glutamine was increasingly inhibited (Figs. 3,4). The relation between the uptake velocity and the external K^+-concentration is logarithmic (Figs. 3, 4, insets) as has to be

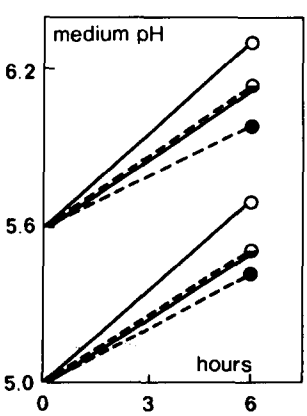
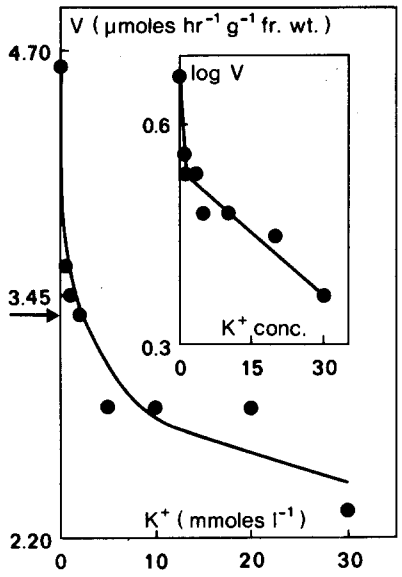


Fig. 2. The effect of light on the proton influx. pH-rises in a medium with (——) and without (— — —) 10 mmol l^{-1} alanine in the light (○) and in the dark (●). The xylem pieces were shaken in 5 mmol l^{-1} citric acid-Na_2HPO_4 buffer solutions.



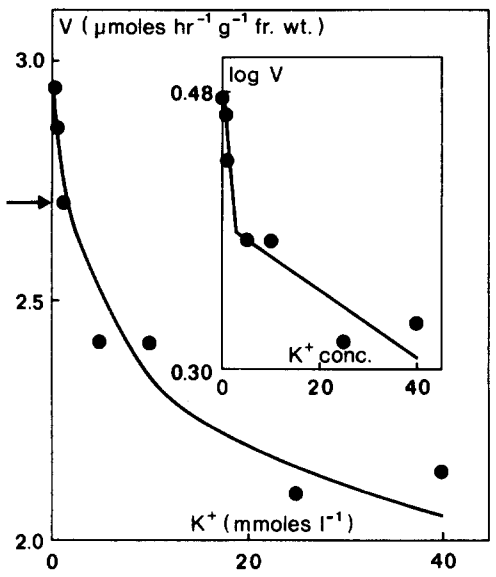


Fig. 3. The effect of the K^+-concentration (KCl) on the alanine (10 mmol l^{-1}) uptake. The uptake time was 7 h. The arrow indicates the uptake rate without K^+ in the medium. Inset: the logarithmic plot of the uptake against the K^+-concentration in the medium.

Fig. 4. The effect of the K^+-concentration (KCl) on the glutamine (10 mmol l^{-1}) uptake. The uptake time was 7 h. The arrow indicates the uptake rate without K^+ in the medium. Inset: the logarithmic plot of the uptake against the K^+-concentration in the medium.

expected from the Goldman equation for the membrane potential. The uptake of alanine and glutamine was stimulated at concentrations lower than 2 mmol l^{-1} K^+ (Figs. 3, 4). The bend in the logarithmic line (Figs. 3, 4 insets) suggests that the stimulation by low K^+-concentrations results from an action on the uptake different from that at high K^+-concentrations.

Dark conditions and the addition of 30 mmol l^{-1} K^+, at the same time, reduced the uptake of alanine to 50% (Table I). The inhibitory action of K^+ and the dark are apparently additive.

The presence of 30 mmol l^{-1} K^+ retarded the pH rise in the media both in

TABLE I

THE EFFECT OF 30 mmol l^{-1} K^+ ON THE AMINO ACID UPTAKE IN THE LIGHT AND IN THE DARK

The uptake of 10 mmol l^{-1} alanine in the light or in the dark in the presence or in the absence of 30 mmol l^{-1} KCl. The uptake time was 7 h. The uptake is expressed in micromoles h^{-1} g^{-1} fresh weight.

	—	30 mmol l^{-1}
Light	2.88	2.24
Dark	1.88	1.43

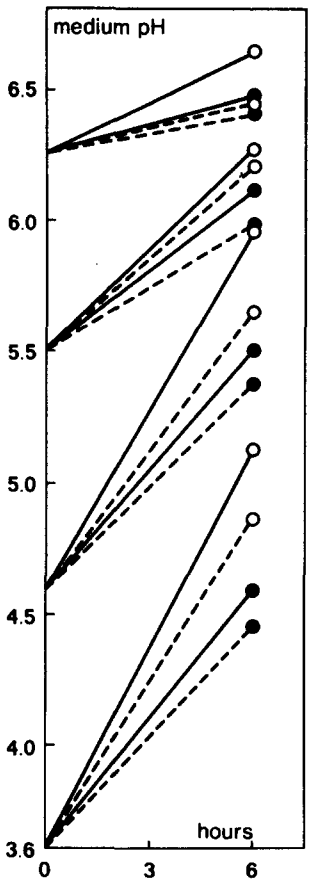


Fig. 5. The effect of a high (30 mmol l^{-1}) K^+-concentration on the proton influx. pH-rises in a medium with 10 mmol l^{-1} alanine in 5 mmol l^{-1} buffer solutions citric acid-Na_2HPO_4 with (— — —) and without (——) KCl in the light (○) and in the dark (●).

the light and in the dark (Fig. 5). In case of a low initial pH, the pH rose more sharply than at a high initial pH (Fig. 5).

DISCUSSION

The present results indicate that the biphasic kinetics in the escape of alanine from the xylem vessels [9] can be attributed to the action of the xylem parenchyma cells. It has been mentioned [9] that the model of Ehwald et al. [8] can explain the biphasic character of the escape from the vessels. This concept, however, does not give a solution for biphasic uptake by cell suspensions [3,5]. On account of the data available at the moment we prefer the model for biphasic uptake advanced by Komor and Tanner [7]. Still, the substrate distribution in the tissue apoplast may be important for the uptake kinetics [8,20] and can emphasize the biphasic nature.

With reference to the model for sucrose uptake by *Chlorella* [7], we have assumed that neutral amino acids are alternatively transported by the protonated or unprotonated form of one carrier system [10]. The protonated form, which is strongly sensitive for the external pH [7], may be identical to the high-affinity system, the unprotonated one to the low-affinity system.

Light may have a dual effect on the amino acid uptake. The high-affinity system may be stimulated by an enhanced proton extrusion, for which the energy can be supplied by the cyclic electron flow in photosynthesis [15,21] or by oxidative phosphorylation [22,23]. The low-affinity system (identical to facilitated diffusion [7,10]) may be stimulated by the light in a different way. Petzold and Jacob [2] already stated that light exerts a direct effect on the plasmalemma permeability. They found that illumination introduced a diffusion-like component in the uptake kinetics, which, obviously, is most recognisable at high external amino acid concentrations [2].

Like in the escape of amino acids from xylem vessels [11], there is evidence for amino acid/proton co-transport into xylem parenchyma cells. In this respect, the K^+-effect on the amino acid uptake seems to be somewhat ambiguous: stimulation of the uptake at low concentrations and inhibition at high concentrations of potassium. The stimulatory effect was ascribed to K^+-co-transport [10], since potassium ions can seemingly replace protons as a co-transporter at an external pH higher than 5.5. But, potassium ions also affect the membrane potential and with increasing K^+-concentrations the depolarisation will proceed. At a certain concentration (in our experiments about 5 mmol l^{-1} K^+) the positive and negative effect of K^+ on the uptake will neutralise each other. If the K^+ concentration exceeds this 'compensation point' the rate of amino acid uptake will decrease (cf. 18,19). The reduction of the co-transport by high K^+ concentrations can be read off from the lower final medium pH in the presence of 30 mmol l^{-1} K^+.

In the light the membrane potential is decreased by proton extrusion [16] and the proton co-transport is intensified. Therefore, in the presence of alanine the final pH was always higher in the light than in the dark.

The results point to amino acid/proton co-transport through the membranes of the xylem parenchyma cells. These cells, and especially the ray cells, are situated in the route leading from the xylem vessels to the sieve tubes. Therefore, co-transport mechanisms may play a role in the xylem-to-phloem transfer of amino acids, formed in the roots of the tomato plant and transported by the xylem in apical direction.

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REFERENCES

1 L. Reinhold, R.A. Shtarkshall and D. Ganot, J. Exp. Bot., 21 (1970) 926.
2 U. Petzold and F. Jacob, Biol. Zentralbl., 94 (1975) 167.

3 J. King, Can. J. Bot., 54 (1976) 1316.
4 A.C. Borstlap, Acta Bot. Neerl., 26 (1977) 115.
5 H.M. Harrington and I.K. Smith, Plant Physiol., 60 (1977) 807.
6 M.S. Blackman and C.S. McDaniel, Plant Sci. Lett., 13 (1978) 27.
7 E. Komor and W. Tanner, J. Gen. Physiol., 64 (1974) 568.
8 R. Ehwald, P. Sammler and H. Goering, Biochem. Physiol. Pflanz., 164 (1973) 596.
9 A.J.E. van Bel, E. Mostert and A.C. Borstlap, Plant Physiol., 63 (1979) 244.
10 A.J.E. van Bel and A.J. van Erven, Planta, 145 (1979) 77.
11 A.J.E. van Bel and A.J. van Erven, Plant Sci. Lett., 15 (1979) 285.
12 A.J.E. van Bel, J. Exp. Bot., 29 (1978) 295.
13 J.L. Bonnemain, 89e Congrès des Soc. Sav. (1964) 315.
14 A.J.E. van Bel, Plant Physiol., 57 (1976) 911.
15 R.M. Spanswick, Can. J. Bot., 52 (1974) 1029.
16 A. Novacky, C.I. Ullrich-Eberius and U. Lüttge, Planta, 138 (1978) 263.
17 A.J.E. van Bel and A.J. van Erven, Z. Pflanzenphysiol., 80 (1976) 74.
18 E. Komor, M. Rotter and W. Tanner, Plant Sci. Lett., 9 (1977) 153.
19 B. Etherton and B. Rubinstein, Plant Physiol., 61 (1978) 933.
20 R. Ehwald, A.B. Meshcheryakov and V.P. Kholodova, Plant Sci. Lett., 16 (1979) 181.
21 C.I. Ullrich-Eberius, A. Novacky and U. Lüttge, Planta, 139 (1978) 149.
22 A. Novacky and A.L. Karr, in: E. Marrè and O. Ciferri (Eds.), Regulation of Cell Membrane Activities in Plants, 1977, pp. 137—144.
23 P.J. Contardi and R.F. Davis, Plant Physiol., 61 (1978) 164.