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POTASSIUM CO-TRANSPORT AND ANTIPORT DURING THE UPTAKE OF SUCROSE AND GLUTAMIC ACID FROM THE XYLEM VESSELS

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SUMMARY

Perfusion experiments with excised internodes of tomato (*Lycopersicon esculentum* cv Moneymaker) showed that the uptake of glutamic acid and sucrose from the xylem vessels is accompanied with coupled proton co-transport and potassium antiport at low pH (<5.5). At high pH (>5.5) both proton and potassium co-transport accompany the uptake. The results fit into the proton pump concept.

INTRODUCTION

Recently we presented a model wherein protons and potassium ions (the latter only at high pH) are co-transported with carrier-substrate complexes across the membranes of tomato internode cells [1]. It may be summarised as follows:.

At low pH (<5.5), protons are pumped out against an electrochemical gradient energised by the conversion of ATP into ADP by membrane-bound ATPase. The high proton-motive force ($\Delta pH + \Delta E$ is high) drives protons, coupled to the carrier-substrate complex, through the membrane into the cells. After release of protons and substrate molecules, the carriers move back to the outer part of the membrane. The K^+ ions leave the cells passively in order to reach their Nernst potential.

At high pH (>5.5), the K^+ ions tend to enter the cells passively, as the membrane potential is more negative than the Nernst potential of potassium ions. The proton-motive force which is smaller than at low pH (as ΔpH is smaller) drives the protons together with the carrier-substrate complex, through the membrane. The K^+ ions compete with the protons for the binding sites of the carrier-substrate complex.

Proton and potassium fluxes can be read off from changes in proton and

potassium content of media in which tissues are shaken [1—4]. In case of perfusion experiments, changes in the proton and potassium content of the perfusate may point to the existence of co-transport mechanisms in cells bordering the translocation path (cf. the experiments of Malek and Baker [5,6]). In internodes, through which amino acids diluted in buffers were perfused, the pH of the vessel fluid is drastically changing along the longitudinal transport route [7]. But as even dead vessel wall material can considerably change the pH of perfusing buffers [8], evidence for proton co-transport cannot be simply concluded from pH changes in the perfusate. Nor is there direct evidence for co-transport given by the appearance of K^+ ions in the perfusate, since K^+ ions in the water free space can be washed out only by water, and secondly, K^+ ions attached to the negatively charged xylem vessel walls can be exchanged against protons supplied by the perfusing amino acid/buffer solutions.

Perfusion of glutamine and glutamic acid through tomato internodes at varying pH-values showed that the escape of these substances is pH-dependent [7]. This was associated with proton co-transport. We aimed to obtain more evidence of the idea that the escape of perfusing sugars and amino acids from the xylem vessels is accompanied by proton co-transport [7,9]. For that purpose, the changes in the pH and the K^+ content of amino acid and sugar solutions, which perfused through excised tomato internodes, were measured.

MATERIALS AND METHODS

Growth and perfusion methods have been described elsewhere [10]. ^{14}C-Labelled solutions (5 mM) of glutamic acid, glutamine or sucrose (in some experiments diluted in buffers) were perfused through excised internodes. Drops of 50 μl were collected at the lower end of the internode. The ^{14}C- and K^+-content and the pH of the drops were alternately measured: the first drop was counted by liquid scintillation spectrometry, the second one was diluted in 2 ml of distilled water and counted by atomic absorption spectrometry, and the third one was measured in a micro-pH meter [2] etc.

The radioactive materials (all universally labelled, 10 mCi/mol) were purchased from the Radiochemical Centre, Amersham, United Kingdom.

RESULTS AND DISCUSSION

Glutamic acid, glutamine or sucrose were perfused through excised tomato internodes. The K^+ and H^+ content of the perfusate was continuously measured. Changes (compared with the background exchange of K^+/H^+ at the cell walls) occurring during perfusion of the substrates may be the consequence of K^+ and H^+ fluxes into and out of the cells along the xylem translocation path. The fluxes may reflect the existence of co-transport mechanisms.

Glutamic acid was chosen for the first experiment because of its low pH,

as the existence of proton co-transport and K^+ antiport should be most distinct at that pH [1,2]. The leakage pattern of glutamic acid corresponds with those earlier found for other amino acids: after 10—20 min of [^{14}C]amino acid perfusion a steady leakage level establishes, during which a constant fraction of the labelled material escapes from the xylem vessels [10,11]. Most of the escaped radioactivity is actively taken up by the cells around the xylem vessels [12,13]. If co-transport is taking place, steady uptake of glutamic acid must be accompanied with a steady decrease of the amount of protons and a steady increase of the K^+ content in the perfusate. The lateral fluxes can be computed by comparing the pH and the K^+ content of

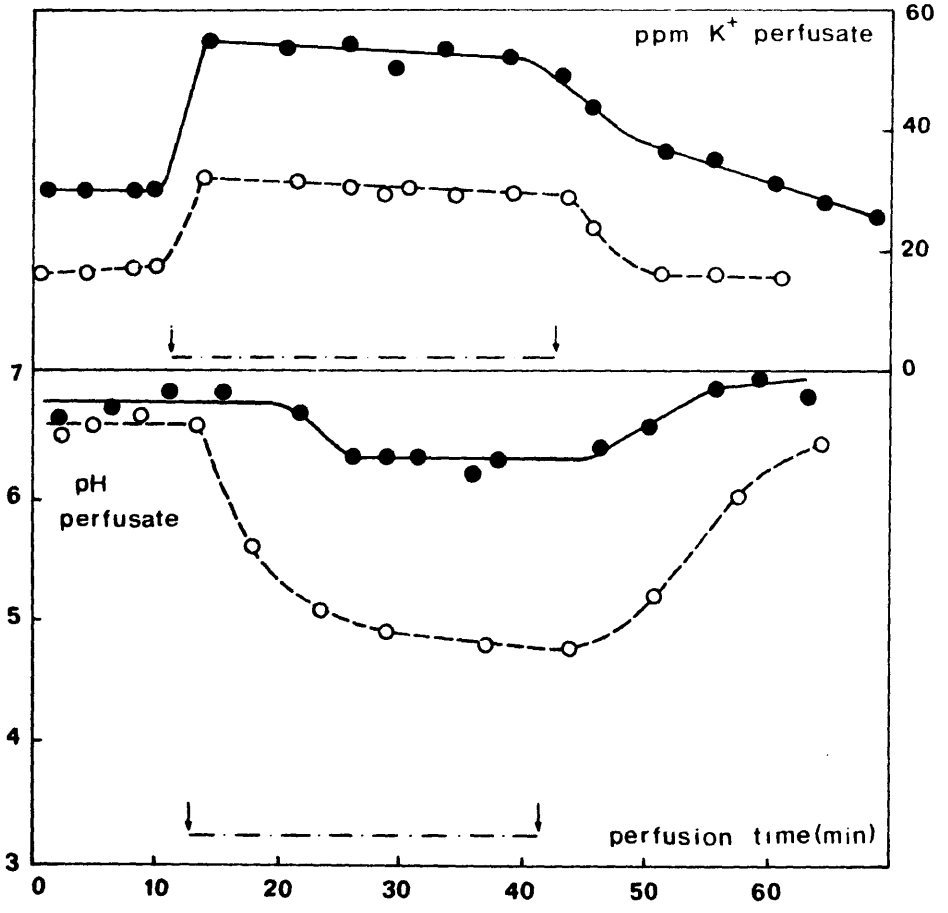

Fig. 1. Changes in the pH and the K^+ concentration in xylem perfusate induced by the uptake of glutamic acid. The lower and upper graphs respectively show the pH and the K^+ content of the perfusate at high (•——•) and low (○——○) temperature. Distilled water (0.3 ml), 0.7 ml 5 mM ^{14}C-labelled glutamic acid (pH 3.3; ↓— · — · —↓) and 0.5 ml distilled water were successively perfused through an internode of 121 mm at 23°C and the pH of the solution perfused was measured. Subsequently, the same procedure was carried out at 5°C. The initial K^+ washed out by distilled water is throught to be coming from the water free space.

the solution supplied at the top of the internode and the perfusate leaking out at the lower end.

After application of 5 mM glutamic acid to the internode at 23°C, the pH of the perfusate decreased to reach a steady level (pH 6.3), but remained far from the pH-value (pH 3.3) applied at the top of the internode (Fig. 1). The rate of proton escape from the xylem vessels decreased at low temperature: at 5°C, the pH of the perfusate fell to 4.8.

The initial K^+ content of the perfusate at 23°C (30 ppm), being the K^+ ions washed out from the water free space, sharply rose to reach a steady level (55 ppm) in response to glutamic acid supply. The release of K^+ into the vessel fluid is also inhibited by low temperature (Fig. 1).

After glutamic acid had been perfused, distilled water was administered and the proton and K^+ content of the perfusate reverted to their initial values (Fig. 1). The results point to coupled proton co-transport and potassium antiport accompanying the uptake of glutamic acid.

To ensure that the proton influx did not result merely from H^+/K^+ exchange in the cell walls, NaOH/phthalate buffers (pH 3.6) with and without Na-glutamate were successively perfused through internodes (Table I). The perfusion procedure was also carried out in the reverse order. In both treatments, the pH increased more during perfusion of the buffer/Na-glutamate solution compared with perfusion of buffer alone. Identical experiments with glutamine and sucrose confirmed this finding. The extra H^+ escape from the xylem vessels was assumed to be the result of proton co-transport. Perfusion of Na_2HPO_4/citrate buffers (pH 4.3) with and without substrates led to the same conclusion (Table I).

TABLE I

THE pH OF XYLEM VESSEL PERFUSATE IN RESPONSE TO PERFUSION OF BUFFERS WITH AND WITHOUT VARIOUS SUBSTRATES

Internodes (55—70 mm) were successively perfused by 2.0 ml 10 mM NaOH/phthalic acid buffer (pH 3.6) and 2.0 ml 5 mM substrate (sucrose, glutamine or Na-glutamate) in 10 mM NaOH/phthalic acid buffer (Na-Ph). The pH of the perfusates are summarised in the first two columns. Experiments carried out in the inverse order yielded the values in columns 3 and 4. The values obtained by successive perfusion of 2.0 ml 10 mM Na_2HPO_4/citric acid buffer (pH 4.3) and 2.0 ml 5 mM substrate in 10 mM Na_2HPO_4/citric acid buffer (Na-Ci) are listed in columns 5 and 6.

	Na-Ph			Na-Ci		
	—substrate	+substrate	+substrate	—substrate	—substrate	+substrate
+/— sucrose	4.8	4.9	5.1	4.7	4.5	4.7
+/— glutamine	4.6	5.2	4.9	4.4	4.5	4.6
+/— Na-glutamate	4.7	4.9	5.1	4.9	4.4	4.6

A linear relation between the pH-change and the internode length was expected, as there is a logarithmic relation between the amount of amino acid escaping along an internode and the internode length [11]. The non-linearity between the pH and the internode length (Fig. 2) may signify that protons are taken up to a minor extent in the lower part of the internode. Simultaneously, the K^+ level in the perfusate remained constant in the lower part of the internode, whereas in the upper part the K^+ content increased with the length (Fig. 3). These results can be explained by referring to the co-transport model for the uptake of organic substances by internode cells [1] as follows:

The pH of the vessel fluid is low near the point of application and we assume that the uptake of glutamic acid is accompanied with proton co-transport and potassium antiport. With increasing pH in the xylem vessels along the internode, the co-transport of protons and the antiport of K^+

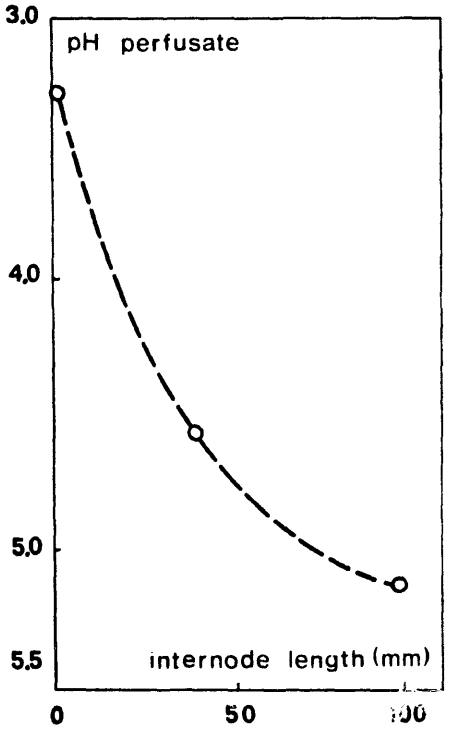

Fig. 2. The longitudinal pH gradient in internode vessels. The xylem vessels of an internode (100 mm) were initially washed with distilled water and, subsequently, perfused with 5 mM ^{14}C-labelled glutamic acid (pH 3.3). The pH of the perfusate was continuously measured. After 10 min the pH of the perfusate reached a steady level and the pH was measured for 30 min. Then a part of the internode was cut off and the remaining internode part (40 mm) was perfused with distilled water until the original high pH had been attained. For 40 min 5 mM [^{14}C]glutamic acid was perfused and the pH attained a new steady level during the last 30 min. The steady pH values of the perfusate are plotted against the corresponding internode length.

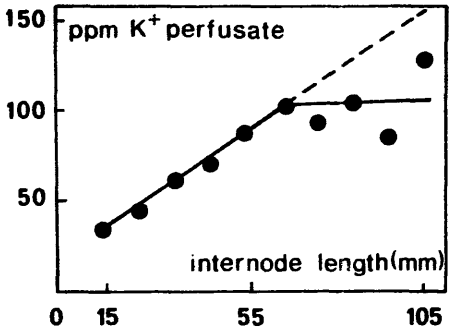

Fig. 3. The longitudinal K^+ gradient in internode vessels during substrate uptake. An internode of 105 mm was perfused with 5 mM [^{14}C]glutamic acid and the K^+ content of the perfusate was continuously measured. When a steady amount of K^+ leaked out, 1 cm of the lower side of the internode was cut off and the remaining piece was perfused with glutamic acid until a new steady K^+ level was measured in the perfusate. Another 1 cm piece was removed and the above procedure was repeated until an internode piece of 15 mm was left. The steady leakage levels of K^+ are plotted against the corresponding length.

decrease and at pH values greater than 5.5 K^+ ions can be even co-transported with the substrates. At pH values greater than 5.5, the Nernst potential for K^+ ions is higher than the membrane potential of the cells around the xylem vessels and K^+ ions tend to move into the cells [14—19].

Perfusion of sucrose (pH 6.8) decreased the K^+ content of the perfusate

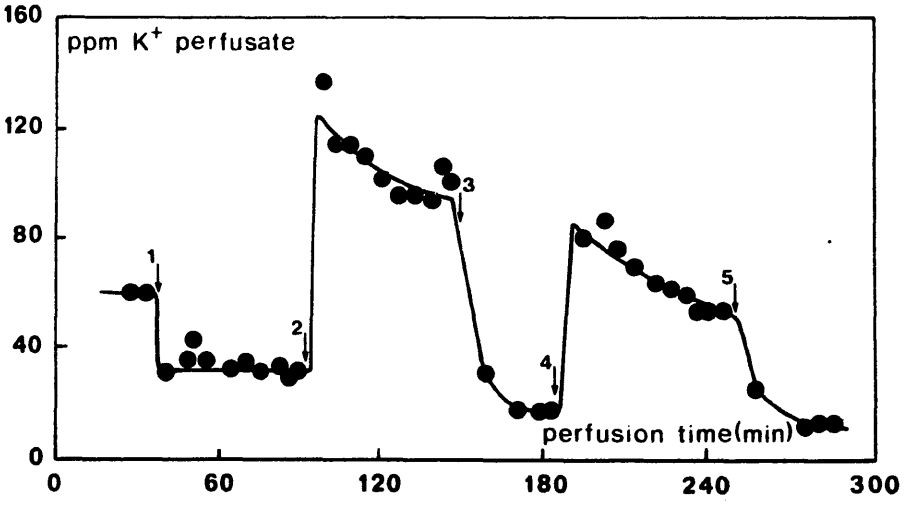

Fig. 4. Potassium fluxes during sugar uptake in relation to the pH of the vessel fluid. Through an internode of 52 mm 0.5 ml distilled water, (↓1) 1.0 ml 5 mM [^{14}C]sucrose (pH 6.8), (↓2) 1.0 ml 5 mM [^{14}C]sucrose in 20 mM phthalic acid/NaOH buffer (pH 3.5), (↓3) 0.5 ml distilled water, (↓4) 1.0 ml phthalic acid/NaOH buffer (pH 3.5), and (↓5) 0.5 ml distilled water were successively perfused. The potassium content of the perfusate was continuously measured under the different treatments.

with 60% in comparison with the perfusion of distilled water. Perfusion of sucrose in phthalic acid/NaOH buffer (pH 3.3) led to a potassium increase in the perfusate of 40% compared with buffer alone (Fig. 4). The decrease of the K^+ washed out by distilled water in the course of this long-term experiment (5 h) is probably due to the exhaustion of the free K^+ content of the xylem vessel walls.

The opposite directions of the K^+ fluxes at different pH values confirm the transition of K^+ antiport at low pH into K^+ co-transport at high pH. The results indicate that the model for co-transport as found in internode disks [1] also explains the events during the uptake of sucrose and glutamic acid from the xylem transport path.

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