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Solute Transport across the Tonoplast of Barley Mesophyll Vacuoles: Mg^{2+} Determines the Specificity, and ATP and Lipophilic Amino Acids the Activity of the Amino Acid Carrier

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Abstract. After stimulation with ATP and in the absence of divalent cations, isolated barley mesophyll vacuoles exhibited massive solute fluxes across the tonoplast, measured either as efflux of endogenous solutes or as uptake of radioactive-labeled compounds. Transported solutes were ions (particularly K^+, NO_3^-, Cl^-) and amino acids (for example, ala, arg, asp, gln, leu, met). Addition of Mg^{2+} in excess of added ATP inhibited fluxes of inorganic ions and of positively charged amino acids, but not, or to a smaller extent, those of neutral amino acids. Thus, Mg^{2+} increased the specificity of the carrier for amino acids such as alanine and glutamine. All ATP-stimulated transport processes were sensitive towards inhibition by lipophilic amino acids, for example by leucine and phenylalanine. After stimulation with sulfhydryl reagents, the inhibitory properties of Mg^{2+} and lipophilic amino acids were lost. These data concur with the hypothesis of a single transporter which exhibits a channel-like structure with a low degree of substrate selectivity in the absence of Mg^{2+}, and which functions as a neutral amino acid carrier in the presence of Mg^{2+}.

Key words: Amino acid transport — ATP (regulation) — Barley vacuoles — Ion transport — Magnesium (effector) — Potassium efflux

Introduction

The large vacuoles of higher plants contain various solutes, predominantly potassium, nitrate and chloride under nonsaline growth conditions. In addition, organic compounds such as sugars, amino acids and alkaloids are also frequently associated with the vacuole of differentiated plant cells. The composition of the vacuolar sap is determined by the selectivity of its surrounding membrane, the tonoplast. Two primary pumps, a number of antiporter systems, carriers for facilitated diffusion, and channels with distinct conductances for ions and organic substrates have been identified (*for a recent review, see:* Martinoia, 1992). However, we still know little about the regulation and the specificity of the transporters. For example, it is yet not clear how many different protein structures are involved in the coordinated trans-tonoplast transport of malate and potassium. Malate is reported to be shuttled across the tonoplast via a selective channel (Smith, Pennington & Pantoja, 1992), a nonselective channel (Hedrich et al., 1988) and a dicarboxylate carrier (Martinoia et al., 1985; Marigo, Bouyssou & Laborie, 1988). A similarly complex situation arises for potassium which may be transported across the tonoplast by an ion channel with low (Hedrich et al., 1988), or high selectivity (Kolb, Köhler & Martinoia, 1987; Klughammer et al., 1992) or possibly by the tonoplast H^+-pumping pyrophosphatase (*see* Rea et al., 1992). In this paper we demonstrate the importance of controlling the composition of the suspension medium of vacuoles, beyond the presence or absence of the substrate of interest, in order to address the physiological function of translocator proteins. Our data suggest that the ATP-stimulated amino acid transporter at the tonoplast (Dietz, Martinoia & Heber, 1989; Dietz et al., 1990; Martinoia et al., 1992) can function as a nonselective transporter carrying cations, anions and amino acids, and is turned into a carrier for neutral amino acids in the presence of excess Mg^{2+}.

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Materials and Methods

Barley (*Hordeum vulgare*, cv. Gerbel) was grown in soil culture in a growth chamber. Primary leaves of 9–11 day-old seedlings were harvested at the beginning of the 14 hr light period. Vacuoles were prepared from protoplast as described by Martinoia et al. (1981) with the following modification: The digestion medium contained 450 mmol liter^{-1} sorbitol, 30 mmol liter^{-1} morpholino ethane sulfonic acid (MES-KOH, pH 5.5), 30 mmol liter^{-1} KCl, 1 mmol liter^{-1} $CaCl_2$, 0.1% (w/v) polyvinylpyrrolidone, 1% (w/v) bovine serum albumin, supplemented with cellulase Y-C (1% w/v), macerocyme (0.25% w/v) and pectolyase Y-23 (0.05% w/v).

Transport Experiments

Uptake and efflux experiments were performed with the silicone oil layer centrifugation technique in principle, as described by Martinoia et al. (1985) and Dietz et al. (1989). The vacuoles were recovered after the last centrifugation step (*cf.* Dietz et al., 1990). Vacuoles (10^7) occupy a volume of about 160 µl, corresponding to a tonoplast area of 0.0307 m^2. For the transport experiments, 400 µl polypropylene tubes were prepared as follows: 40 µl of the vacuolar suspension was added to 60 µl of an osmotically adjusted solution, so that the final concentrations were: 40% Percoll, 30 mmol liter^{-1} K gluconate, 30 mmol liter^{-1} HEPES, KOH, pH 7.5, 2 mmol liter^{-1} dithiothreitol and 0.5% bovine serum albumin. DTT and BSA were omitted in the experiments with sulfhydryl reagents such as *p*-chloromercuriphenyl sulfonic acid (pCMBS). All other reagents were added as indicated in the legends. The incubation reaction was overlayered with 150 µl silicone oil (AR 200, Wacker Chemie, Munich, Germany) and 60 µl H_2O. The transport was terminated by centrifugation for 30 sec. During this centrifugation, intact vacuoles floated through the silicone oil layer to the upper H_2O phase which was then used for cation, anion or amino acid determinations and for measuring the activity of the vacuolar marker enzyme α-mannosidase (Boller & Kende, 1979). For the measurement of amino acid uptake, ^{14}C-labeled amino acids were added to the incubation reaction at an activity of 4–5 kBq; the final amino acid concentration was 1 mmol liter^{-1}; ^3H_2O was included to determine the volume of the recovered vacuoles (*cf.* Martinoia et al., 1985).

Analysis of the Vacuoles

Amino acid and anion composition of the recovered vacuolar fraction was determined with an anion chromatograph (Schröppel-Meier & Kaiser, 1988) or amino acid analyzer (Dietz et al., 1989). Cation contents of the vacuolar fractions were analyzed with an induced coupled plasma atomic emission spectroscope (*Jobin Yvon JY 70, Instruments, France*) (Dietz et al., 1992).

Results

Solute Contents of Isolated Barley Vacuoles

The isolation of barley vacuoles was performed in media of an osmolarity close to 600 mosmol liter^{-1}. Since vacuoles are not a turgor system, the osmolarity of the surrounding medium and the vacuoles must be equal. Table 1 shows the concentrations of inorganic ions and amino acids in isolated vacuoles. The mean sum of these compounds was 727 mmol liter^{-1}. Although not all minor constituents of the vacuoles were measured, molecular interaction, in particular between divalent cations, inorganic anions and organic acids, provided a plausible explanation of why the measured concentrations exceeded the osmolarity. Measurements of solute fluxes require standardization. For ion flux experiments, we found it most reliable to relate the measured concentrations to the vacuolar phosphate contents which proved to be unchanged during the experiment. The mean phosphate content was 437 ± 74 µg 10^{-7} vacuoles (n = 9). After an incubation for 47 min, the mean phosphate contents of the reisolated vacuoles had decreased by only 1.5 µg 10^{-7} vacuoles, i.e., by less than 0.4%.

Table 1. Solute contents of isolated mesophyll vacuoles in mmol liter^{-1}

Vacuolar solutes	Concentration [mmol liter^{-1}]
Cations	
K^+	294.6 ± 43.2
Na^+	19.4 ± 4.9
Mg^{2+}	5.4 ± 1.2
Ca^{2+}	1.5 ± 0.5
Anions	
Cl^-	123.8 ± 25.0
NO_3^-	92.1 ± 34.6
PO_4^{3-}	92.1 ± 23.3
SO_4^{2-}	6.3 ± 1.8
Malate	15.1 ± 6.4
Amino acids	76.7 ± 38.4
Total solute concentrations	727.1 mmol liter^{-1}

The data are means (±SD) of at least 20 determinations.

Potassium Efflux from Isolated Vacuoles

ATP was shown previously to stimulate the rate of amino acid transport across the tonoplast membrane (Dietz et al., 1989, 1990; Martinoia et al., 1992). Therefore, ATP was tested for its effect on potassium efflux from isolated vacuoles (Fig. 1). Even in the standard medium or in medium supplemented with EDTA, efflux of potassium was observed. Upon addition of ATP, the efflux increased by a factor of about 2.5. Addition of EDTA had no effect on ATP stimulation. A similar increase in potassium efflux was observed in the presence of the nonhydrolyzable ATP-analogue adenylylimidodiphosphate (Ado*PP*[NH]*P*). Addition of excess

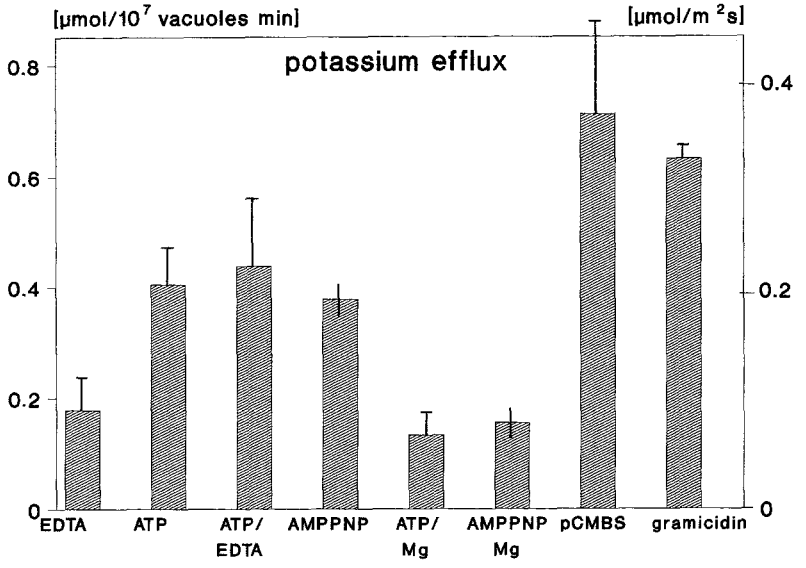

Fig. 1. Potassium efflux from isolated barley vacuoles as affected by different compounds. The incubation time was 20 min. The effector concentrations were: EDTA: 1 mmol liter^{-1}; ATP, AdoPP[*NH*]P: 5 mmol liter^{-1}; Mg(gluconate)$_2$: 10 mmol liter^{-1}; pCMBS: 1 mmol liter^{-1} and gramicidin: 1 µmol liter^{-1}. The numbers of independent experiments were in the order as shown: 3, 4, 12, 2, 14, 2, 12, 3. The maximum standard deviation σ_{n-1} is shown for all experiments with at least three replicates; for the two experiments with AdoPP[*NH*]P, the region of the two results is indicated.

Mg^{2+} caused an inhibition of the ATP and Ado*PP*[NH]*P*-stimulated potassium efflux to the level observed in the absence of ATP. Maximum rates of potassium efflux were measured in the presence of pCMBS. They were similar to the rates seen after incorporation of the potassium channel-forming exotoxin gramicidin into the tonoplast, and somewhat higher than after addition of a mixture of valinomycin and nigericin (*data not shown*).

Anion Efflux

Chloride and nitrate were the predominating anions in barley vacuoles. Although their mean concentrations were similar or the chloride concentration was even higher than that of nitrate, nitrate was lost with about twice or more the initial rate of chloride, confirming results by Lew and Spanswick (1985) who used fluorescence quenching to determine the relative permeability of the tonoplast for various ions. Only after stimulation with pCMBS, efflux rates were similar for NO_3^- and Cl^- (*results not shown*). Addition of ATP stimulated efflux of NO_3^- by a factor of more than 2. After ATP stimulation (set to 100%), Mg^{2+} decreased efflux of NO_3^- to 30 ± 12% ($n = 4$). The Mg^{2+}-dependent inhibition of anion efflux is shown in more detail in Fig. 2. The chloride and nitrate contents of the vacuoles were determined after 2, 11 and 20 min. Largest efflux was seen in the presence of valinomycin and nigericin. Interestingly, the kinetics of anion loss was complex: In the first phase, nitrate was lost at a higher rate than in the second phase, whereas chloride efflux was low in the first and increased in the second phase of the experiments. Both chloride and nitrate loss was low under control conditions; it was stimulated 3.5-fold in the presence of 5 mmol liter^{-1} ATP. Addition of Mg^{2+} at a concentration of 2.5 mmol liter^{-1} had only a slight inhibitory effect, whereas addition of excess Mg^{2+} decreased the ATP-stimulated efflux to the control rates.

Transport of Amino Acids

The stimulatory function of ATP and ATP-analogues on amino acid transport has been described previously. Both efflux of endogenous amino acids (Dietz et al., 1989) and uptake of externally added ^{14}C-labeled amino acids such as ala, gln, leu (Dietz et al., 1990) or asp and arg (Martinoia et al., 1992) have been shown to be activated by ATP. These observations are confirmed by the data shown in Fig. 3. It has been shown previously that uptake of amino acids was linear with time (Dietz et al., 1990). Therefore, rates were determined from standard incubation times of 2′ and 20′. Uptake of the ^{14}C-labeled amino acids ala and arg was stimulated after addition of ATP. However, it was a new finding that ala and arg uptake were differentially affected by Mg^{2+}. Whereas uptake of arginine was inhibited to the level of the unstimulated control, Mg^{2+} had no significant effect on ATP-stimulated alanine transport. The same inhibitory effect of Mg^{2+} was also observed for K^+ efflux (Fig. 1). A more detailed analysis showed a similar Mg^{2+}-dependent inhibition of K^+ efflux and arg uptake (*data not shown*). Half-maximal inhibition of K^+ efflux was observed at a Mg^{2+} concentration of 3.5 mmol liter^{-1}, as compared to 3 mmol liter^{-1} Mg^{2+} necessary for 50% inhibition of ATP-stimulated arg uptake (Martinoia et al., 1992). Table 2 summarizes the differential effect of Mg^{2+} on transport of amino acids and ions. Excess Mg^{2+} inhibited ATP-stimulated asp, arg, K^+, NO_3^- and Cl^-

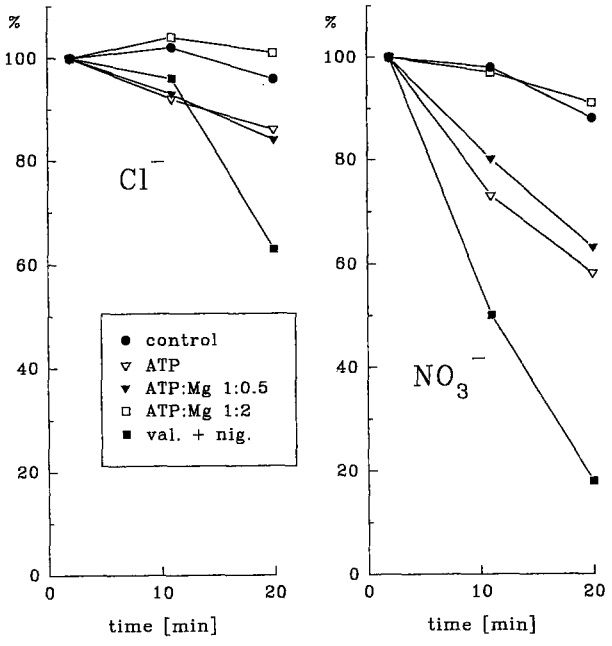

Fig. 2. Nitrate and chloride efflux from isolated vacuoles. Vacuoles were incubated in standard medium supplemented with effectors as indicated. The vacuolar nitrate and chloride contents were determined at time points 2, 11 and 20 min. The data are mean values of two experiments with similar results (maximum deviation 11%). The initial anion contents (set to 100%) differed for the two experiments: the endogenous chloride concentrations were 127 and 144 mmol liter^{-1}, respectively, and the nitrate concentrations 67 and 57 mmol liter^{-1}, respectively. Effector concentrations were: ATP: 5 mmol liter^{-1}, Mg(gluconate)$_2$: 2.5 and 10 mmol liter^{-1}, valinomycin: 1 μmol liter^{-1}, nigericin: 10 μmol liter^{-1}.

transport to a level close to the unstimulated control, whereas ala, phe and gln showed no, or a much lower degree, of inhibition.

Leucine-Inhibition of ATP-Activated Transport

As depicted in Fig. 4 (*Top*), leu inhibited not only uptake of amino acids but also efflux of K^+. In contrast to lipophilic amino acids such as leu and phe (*cf.* Dietz et al., 1989), gln showed no effect on transport of amino acids or ions. All tested transport activities were highly stimulated by pCMBS and NEM. After pCMBS-stimulation, leucine and Mg^{2+} lost their inhibitory function (Table 3). The effect of leu on phe uptake was similar for the ATP/EDTA and ATP/Mg^{2+}-activated transport (Fig. 4, *Bottom*). However, no further inhibition by leu was seen after Mg^{2+} inhibition of ATP-stimulated K^+ transport.

Discussion

The Vacuole as Storage Compartment

The tonoplast is of critical importance for establishing and maintaining specific concentration gradients between the cytosol and the vacuole. Under conditions of high ion concentrations in the rooting medium and in the absence of stringent exclusion mechanisms in the roots, excessively accumulating ions will be compartmentalized into the vacuoles. Ions such as Cl^-, NO_3^- and Na^+ are accumulated to vacuolar concentrations higher than in the cytoplasm (Martinoia et al., 1986). The situation is different for N and C assimilates which are mainly exported to sink tissues via the phloem. Consequently, sugar and amino acid concentrations are usually higher in the cytoplasm. Nevertheless, under conditions of restricted phloem loading, they also accumulate in the mesophyll and are necessarily, for osmotic reasons, partly deposited inside the vacuole.

The Substrate Specificity of the Tonoplast Amino Acid Carrier

The ATP-activated amino acid carrier has been proposed to catalyze the transfer of amino acids across the tonoplast for intermediate storage of amino acids inside the vacuole (Dietz et al., 1990; Goerlach & Willms-Hoff, 1992) and to allow for amino acid release from the vacuole when the cytoplasmic amino acid concentrations fall below the vacuolar concentrations. Efflux studies have shown that the transporter carries all amino acids which endogenously accumulate in vacuoles (Dietz et al., 1989). The data shown above demonstrate the even broader spectrum of substrates of the ATP-activated transporter. Inorganic cations and anions are rapidly transferred across the tonoplast after activation with ATP in the absence of Mg^{2+}. There is, however, a cutoff of molecular mass above which solutes are no longer transported. The tripeptide glutathione is neither transported in the presence of ATP/EDTA nor in the presence of MgATP, whereas oxidized glutathione (GSSG) is taken up by isolated vacuoles only after addition of ATP in the presence of Mg^{2+}, indicating energized transport (Dietz et al., 1992). A similar broad substrate specificity has been reported for the dominant tonoplast ion channel, the so-called SV-type channel (Hedrich et al., 1988). It is evident from the above arguments that both tonoplast transporters, the SV-type ion channel and the ATP-stimulated solute carrier, must be subjected to strict control to prevent nonspecific dissipation of the transtonoplast solute gradients. Although controls beyond energization of transport processes by ATP have frequently been described in animal systems, this does not seem to be a frequently used principle in plants. In connection with the similar effects of lipophilic and hydrophilic amino acids and of Mg^{2+} on the transport of ions and amino acids, we find it unjustified to assume the existence of more than one ATP-regulated transporter at the tonoplast right now.

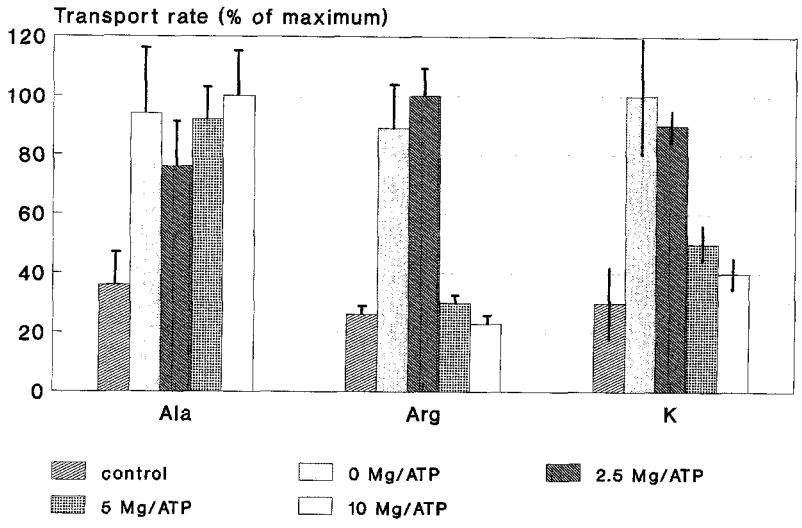

Fig. 3. Effect of increasing Mg concentrations on efflux of K^+ and uptake of ala and arg by isolated vacuoles. Maximum transport rates were set to 100%, which allows comparison of the transport rates; 100% corresponded to 1.58 ± 0.43 nmol $(10^7$ vacuoles $min)^{-1}$ for ala, 3.83 ± 0.12 nmol $(10^7$ vacuoles $min)^{-1}$ for arg and for K^+ 0.58 μmol $(10^7$ vacuoles $min)^{-1}$. Amino acid transport was measured at 1 mmol $liter^{-1}$ concentrations of arg and ala; the endogenous K^+ concentration was close to 300 mmol $liter^{-1}$ (*cf.* Table 1). The control rates were determined in standard transport medium. ATP (5 mmol $liter^{-1}$) was added to all other incubations in the presence of varying Mg^{2+} concentrations, i.e.; 0, 2.5, 5 and 10 mmol $liter^{-1}$ $Mg(gluconate)_2$. The original data were obtained from 8 (ala), 4 (arg) and 2 (K^+) measurements (the bars connect the two results). At 10 mmol $liter^{-1}$ Mg, ala uptake was 1.68 ± 0.27, arg uptake 0.90 ± 0.05 nmol $(10^7$ vacuoles $min)^{-1}$ and K^+ efflux 0.21 and 0.26 μmol $(10^7$ vacuoles $min)^{-1}$, respectively.

Mg^{2+} as Regulator of the Tonoplast Amino Acid Carrier

The data of this communication suggest that Mg^{2+} is an important regulator of the ATP-stimulated amino acid transporter. Inhibitory properties of Mg^{2+} on transporters have been reported: The megachannel from rat liver mitochondria (Szabo & Zoratti, 1992) and the anion channel of the inner mitochondrial membrane of animals (Beavis, 1992; Kinnally, Antonenko & Zorov, 1992) are inhibited by increasing Mg^{2+} concentrations. In addition to the inhibitory effects, Mg^{2+} seems also to exert regulatory functions in respect to the specificity of the vacuolar transporter. At high Mg^{2+} concentrations, the ATP-stimulated transport of anions and cations, and of amino acids with positive or negative net charge was suppressed to the level of the unstimulated control, whereas uptake of ala was hardly and that of gln and phe only partly affected. The free concentration of Mg^{2+} in the cytoplasm of plant cells has been measured by Mg^{2+}-selective microelectrodes and has been reported to be in the range of 0.7 to 1.5 mmol $liter^{-1}$ (Thaler, 1991). It has to be concluded that Mg^{2+} is usually present at sufficient high free cytoplasmic concentrations to saturate the transporter with Mg^{2+} and thereby to modulate its substrate preference towards a specificity for amino acids.

Lipophilic Amino Acids as Inhibitors of Amino Acid Transport

The ATP-modulated transporter is subjected to further regulation by lipophilic amino acids. As reported previously (Dietz et al., 1989, 1990; Martinoia et al., 1992),

Table 2. Effect of Mg^{2+} on ATP-stimulated solute fluxes

	Ala	Phe	Gln	NO_3^- (%)	Asp	Arg	Cl^-	K^+
S_R	103	70	58	17	14	12	11	−17

Transport of amino acids was measured as uptake of radioactive label, transport of K^+, NO_3^- and Cl^- as efflux as described above. The values give the mean remaining stimulation of transport (S_R) in the presence of 5 mmol $liter^{-1}$ ATP and 10 mmol $liter^{-1}$ Mg^{2+} ($T_{Mg/ATP}$) as a percentage of the stimulation after addition of 5 mmol $liter^{-1}$ ATP (T_{ATP}) as related to the control rate (T_C):

$$S_R = 100(T_{Mg/ATP} - T_C)(T_{ATP} - T_C)^{-1}.$$

lipophilic amino acids such as leu, val and phe are potent inhibitors of the ATP-stimulated amino acid carrier. Three observations suggest that this effect of lipophilic amino acids is not due to competitive inhibition of the transporter: (i) Leu has been shown to inhibit both efflux and influx of amino acids regardless of the endogenous concentrations of lipophilic amino acids. (ii) Hydrophilic amino acids, such as gln which are also transported at high rates and which therefore should be similarly effective competitors, revealed much lower inhibitory properties than lipophilic amino acids. This differential effect of leu *vs.* gln was also seen after reconstitution of the transport system by incorporation of solubilized tonoplast proteins into liposomes (Thume & Dietz, 1991). (iii) Over a wide range of substrate concentrations, the transport rate was roughly linearly related to the substrate concentrations (Dietz et al., 1990; Martinoia et al., 1992). Therefore, only small competitive interactions should be expected between

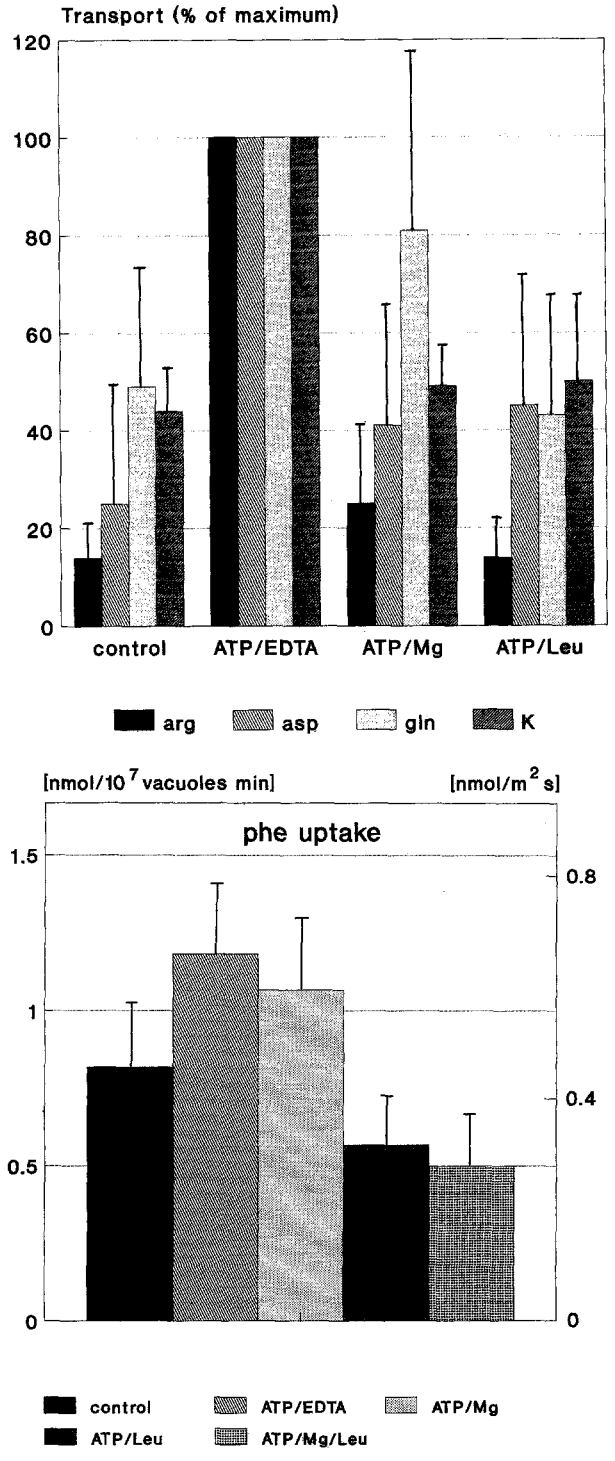

Fig. 4. Leucin-inhibition of ATP-stimulated transport. (*Top*) Comparison of the effects of ATP/EDTA, ATP/Mg and ATP/EDTA + leucin (10 mmol liter^{-1}) on arg, asp and gln uptake and K^+ efflux. Amino acid concentrations were 1 mmol liter^{-1}. The data are mean values (±SD) of 5 (arg, asp and gln) measurements with 3–4 replicates and 3 measurements (K^+), respectively. To normalize the scale of the y-axes, maximum transport values in the presence of ATP/EDTA were set to 100%. (*Bottom*) Effect of leu (10 mmol liter^{-1}) on ATP/EDTA- and ATP/Mg-stimulated uptake of phe by isolated vacuoles.

Table 3. Loss of Mg^{2+}- and leu-dependent inhibition of K^+ efflux of isolated vacuoles after stimulation with pCMBS

Incubation condition	Efflux of vacuolar K^+ [µmol (10^7 vacuoles min)$^{-1}$]	*n*	%
Effect of leu			
ATP/EDTA	0.47 ± 0.12	8	100
ATP/EDTA + leu	0.25 ± 0.06	3	53
ATP/EDTA + gln	0.43 ± 0.15	4	91
ATP/Mg^{2+}	0.27 ± 0.10	3	100
ATP/Mg^{2+} + leu	0.24 ± 0.13	3	89
pCMBS	0.82 ± 0.22	3	100
pCMBS + leu	0.81 ± 0.27	3	99
Effect of pCMBS			
ATP/EDTA	0.43 ± 0.18	6	100
ATP/Mg^{2+}	0.16 ± 0.06	6	37
ATP/Mg^{2+} + pCMBS	0.67 ± 0.21	6	156

Isolated mesophyll vacuoles were incubated in the presence of the effectors as indicated: The concentrations were: ATP: 5 mmol liter^{-1}, $Mg(gluconate)_2$: 10 mmol liter^{-1}, leu or gln: 10 mmol liter^{-1}, pCMBS: 1 mmol liter^{-1}. Incubation was terminated by flotation after 20 min, and potassium contents of the reisolated vacuoles were determined. The initial K^+ content of the vacuoles was 50.4 ± 8.1 µmol (10^7 vacuoles)$^{-1}$.

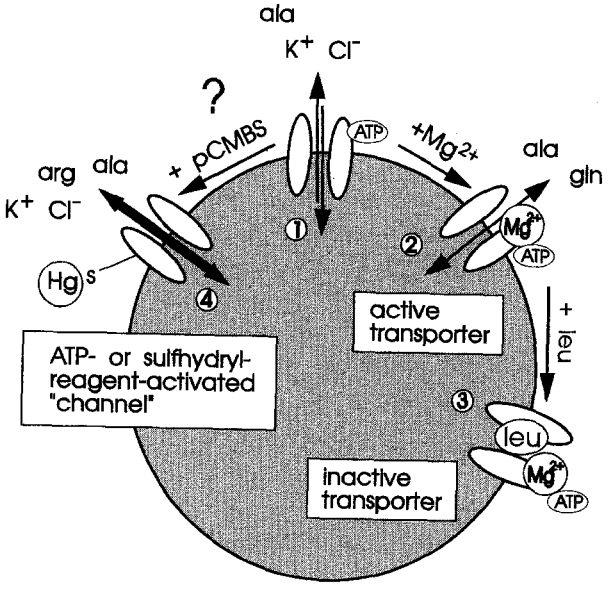

Fig. 5. Schematic representation of the hypothetically proposed four activation states of the tonoplast amino acid carrier: The ATP-activated (*1*) and the pCMBS-modified protein (*4*) show a broad substrate specificity and high transport rates ("channel"-like). It should be noted that there is only some indirect evidence that the large fluxes observed in the presence of pCMBS and NEM are due to SH-group modification of the amino acid carrier. Addition of Mg^{2+} to the ATP-activated state causes the transporter to become more specific for certain neutral amino acids (*2*). The ATP-activated or MgATP-modulated amino acid transporter is inhibited in the presence of lipophilic amino acids (*3*).

the various substrates below the apparent K_M values. In contrast to this expectation, the K_I (leu) for efflux of endogenous amino acids was below 2 mmol liter^{-1} (Dietz et al., 1989), and the K_I (phe) for arginine uptake was about 1 mmol liter^{-1}. Since additive whole leaf concentrations of lipophilic amino acids (ile + leu + met + phe + val) are in the range of 1 mmol liter^{-1} and higher (*cf.* Dietz, 1989) and based on their preferential cytoplasmic compartmentation, it has to be concluded that the ATP-stimulated amino acid carrier is in the inhibited state under normal conditions (Fig. 5). It is tempting to speculate that the transporter is activated by a decrease in the concentration of cytoplasmic lipophilic amino acids under specific metabolic conditions allowing the release of stored vacuolar amino acids. A decrease in cytoplasmic amino acid concentration will occur when efficient amino acid export via the phloem is coupled to limited nitrogen assimilation—for example, under nitrogen deficiency. Furthermore, the inhibitory effect of lipophilic amino acids on ATP-stimulated ion and amino acid transport may be taken as coincidental evidence that both kinds of transport are mediated by the same carrier.

A Hypothetical Model of Three (or Four) Distinct States of the Tonoplast Amino Acid Carrier

The four proposed activity states are summarized in Fig. 5. In addition to the ATP- and pCMBS-activated states, which both have a broad substrate specificity but are unlikely to occur in vivo, the transporter switches between an active and inactive state mainly as a function of the cytoplasmic lipophilic amino acid concentration. Artificial transformation of carriers to pore-like proteins after chemical modification with SH-group modifiers has been reported for the mitochondrial aspartate/glutamate- and ADP/ATP-carrier (Dierks et al., 1990; Dierks, Salentin & Kramer, 1990). However, it should be mentioned that, besides the correlations regarding the specificity of the efflux, there exists no strong evidence that the observed stimulation of fluxes after addition of sulfhydryl reagents is caused by chemical modification of the amino acid carrier.

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