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Cortical Cell Fluxes and Transport to the Stele in Excised Root Segments of *Allium cepa* L.

III. Magnesium

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Summary. From compartmental analysis of radioisotope elution measurements, concentrations and fluxes of Mg^{2+} were estimated for cortical cells in root segments of onion, *Allium cepa* L., relative to a complete nutrient solution containing 0.25 mM Mg^{2+}. Five compartments for Mg^{2+} in the cortex were found and, in order of increasing rates of exchange, identified with the vacuoles and the cytoplasm of the cortical parenchyma, the Donnan free space, the water free space, and the superficial film of solution on the segments. With the Ussing-Teorell flux ratio equation as the criterion, it was concluded that Mg^{2+} entered the cytoplasm passively and was actively pumped back across the plasmalemma. Mg^{2+} concentration in the vacuole could be estimated only as lying between wide limits (1.3 to 14.3 μeq ml^{-1}), but whatever the concentration within this range, it was concluded that Mg^{2+} was passively distributed across the tonoplast. Net flux was zero and the vacuolar concentration commensurate with this was found to be 6.6 μeq ml^{-1}. The transported fraction of total efflux, appearing at the segment cut ends, was estimated separately. Magnesium was found to be transported almost exclusively in the basipetal direction.

Introduction

The characteristics of Mg^{2+} absorption have received little attention beyond a few studies of net uptake by whole plants or excised roots (Moore *et al.*, 1961; Lazaroff and Pitman, 1966; Leggett and Gilbert, 1969; Maas and Ogata, 1971). These authors, and Kaplan (1969) in a kinetic study using radioactive Mg^{2+}, concluded that Mg^{2+} absorption was metabolically mediated, at least in part. However, on those occasions when electrochemical potential evidence from higher plants has been considered, it has been concluded that Mg^{2+} enters the plant down a diffusion gradient, and that it fails to reach equilibrium concentration due either to low membrane permeability or to an efflux pump (Bowling *et al.*, 1966; Higinbotham *et al.*, 1967). From studies of ion content and membrane potentials in non-vacuolated fucoid eggs, Allen *et al.* (1972) and Weisenseel and Jaffe (1972) concluded that a Mg^{2+} efflux pump must be necessary to maintain the low internal concentration, relative to sea water.

In this paper, the individual fluxes of Mg^{2+}, both into and out of cytoplasm and vacuole, are estimated from compartmental analysis of radioactive Mg^{2+} uptake and elution data, and it is conluded that, in view of the electrochemical activity ratio between the cytoplasm and outside solution, Mg^{2+} entry into onion root cortex cells is indeed passive, and is controlled by an efflux pump at the plasmalemma.

Materials and Methods

Roots were grown from bulbs of an Egyptian main crop variety of *Allium cepa* L., and used to provide 4 cm segments taken from the zone 0.5–4.5 cm behind the root tip. The roots were grown and segments subsequently incubated and eluted in a solution of the following composition (in mmol/l): $Ca(NO_3)_2$, 1.0; KCl, 1.0; $MgSO_4$, 0.25; NaH_2PO_4, 0.904; Na_2HPO_4, 0.048 (pH 5.6). One per cent sucrose was incorporated in the solution used for uptake and elution.

For uptake measurements and loading with radioisotope prior to elution, the segments were incubated in groups of 7 in 125 ml flasks containing 25 ml of the experimental solution. The flasks were shaken at 80 oscillations per min and 25° C.

Radioactive magnesium, obtained from the Brookhaven National Laboratory, Upton, N.Y., U.S.A., was incorporated into the nutrient solution at an initial level of about 20 μCi per flask for loading prior to elution, and at an initial level of about 2 μCi per flask for uptake measurements. The activity was supplied as $^{28}MgCl_2$ at a concentration of 6 μeq Mg per ml in dilute HCl, pH adjusted to 4.1 with NaOH. This was found to give a sodium concentration of about 17 μeq per ml in the solution as supplied. Adjustments were made to the non-radioactive Mg^{2+}, Na^+ and Cl^- components of the labelled solutions, as appropriate.

Elution of activity from the segments with non-radioactive solution was carried out in a 3-section perspex chamber which allowed estimation of surface efflux separately from that at the cut ends of the segments. In each experiment, a parallel sample was eluted in a shallow based funnel, shaken to simulate flask uptake condi-

Table 1. Efflux constants (k), half-times for exchange ($t_{\frac{1}{2}}$) and apparent contents for Mg^{2+} in each compartment, and apparent influx to the vacuole (I_v/t_{up})

Compartment	k (s^{-1})	$t_{\frac{1}{2}}$ (min)	Apparent content ($\mu eq\ g^{-1}$ fresh wt.)	I_v/t_{up} ($\mu eq\ g^{-1}\ h^{-1}$)
Vacuole: S[a]	2.70×10^{-6}	4283	11.2	0.050
Vacuole: T	3.96×10^{-6}	2919	11.1	0.052
Cytoplasm	1.55×10^{-4}	74	0.32	
Donnan free space	8.37×10^{-4}	14	0.25	
Water free space	3.55×10^{-3}	3.2	0.05	
Superficial	3.84×10^{-2}	0.30	0.23	

[a] S, results from counts *via* the segment surface only.
T, results from counts from both surface and cut ends.

Table 2. Unidirectional fluxes and contents of Mg^{2+} computed form the results in Table 1, using the relationships given by Macklon (1975a)

Compartment	Influx ($\mu eq\ g^{-1}\ h^{-1}$)	Efflux ($\mu eq\ g^{-1}\ h^{-1}$)	Content ($\mu eq\ g^{-1}$)
Vacuole (max)[a]	0.081	0.14	11.2
Vacuole (min)	0.053	0.013	1.0
Cytoplasm (max)[a]	0.23	0.29	0.66
Cytoplasm (min)	0.23	0.19	0.43

[a] Two sets of data are given, one based on the estimated maximum "free" Mg^{2+} content of the vacuole, and one based on an estimate of minimum "free" content.

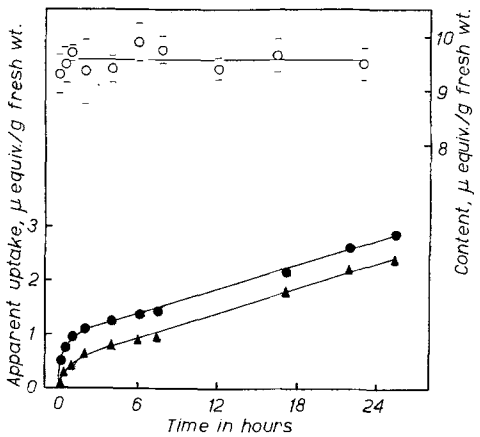

Fig. 1. Apparent uptake (left-hand ordinate) and net uptake (right-hand ordinate) of Mg^{2+} as a function of time. ●—● total apparent uptake; ▲—▲ after desorption in unlabelled solution at 0° C for 30 min; ○—○ total Mg^{2+} content. Horizontal lines indicate ± the standard error of the mean (5 values)

tions. Fuller details of the apparatus and procedures used are given in an earlier paper (Macklon, 1975a).

Root samples were ashed, taken up in 6 N HCl, and made to volume with 1% HCl. Part of this extract was used for radioassay. An aliquot of each sample of segment digest or efflux washing solution was mixed with an equal volume of "Instagel" scintillator and counted using a Packard "Tri-Carb" liquid scintillation spectrometer. Total magnesium was determined on the remainder of the same segment extracts by atomic absorption spectrophotometry, after the radioactivity had decayed.

Results

Tissue was allowed to take up radioisotope for 17 h and elution of $^{28}Mg^{2+}$ was measured over the subsequent 10 h. From counts of activity remaining in the tissue at the end of elution and in each change of washing solution, an efflux curve was constructed. Analysis of the results using semi-logarithmic plots, in the manner described earlier (Macklon, 1975a), resolved the efflux curve into 5 linear phases. As with Ca^{2+} (Macklon, 1975b), the 5 components of the compound efflux curve are equated, in order of decreasing half-times of exchange ($t_{\frac{1}{2}}$), with cell vacuole, cytoplasm, Donnan free space, water free space and superficial film of solution. Primary efflux data are presented in Table 1. Separate estimation of stelar leakage from the segment cut ends showed that vacuolar efflux was otherwise over-estimated, but none of the other compartment constants were affected. Values obtained from funnel elution experiments were not significantly different from those obtained for "total" efflux into the 3-section chamber.

Real fluxes and contents of Mg^{2+}, calculated from the apparent values of Table 1 using the equations quoted by Macklon (1975a), are given in Table 2. Two sets of values are shown. One is based on the maximum magnesium content of the vacuole (11.2 $\mu eq\ g^{-1}$) calculated on the assumption that all the magnesium not identified as exchangeable in the cytoplasm and free space was in ionized form in the vacuole. The second set of values given in Table 2 is based on an estimate of the minimum vacuolar content, taken as the product of apparent influx and loading time. This estimate (1.0 $\mu eq\ g^{-1}$) would imply that a large proportion of magnesium in the tissue was in a non-exchangeable and non-ionized form either in the vacuole, or in the cytoplasm and wall.

Net uptake of Mg^{2+} during loading and elution was negligible, and apparent influx was linear after the first 2 or 3 h (Fig. 1). As with Ca^{2+} (Macklon, 1975b), the uptake shoulder was reduced but not eliminated by 30 min desorption in unlabelled solution at 0° C.

Using the cell compartment volumes reported previously (Macklon, 1975a), the content values given in Table 2 were used to calculate cytoplasm and

Table 3. Chemical concentrations (C_j^i) and activities[a] (α_j^i) of Mg^{2+} inside each membrane, i.e. in the cytoplasm and vacuole, of root cortex cells, and the flux and electrochemical activity ratios across plasmalemma and tonoplast

Vacuolar Mg^{2+} content[b]	Permeability barrier	C_j^i (μeq ml^{-1})	α_j^i (μeq ml^{-1})	J_{in}/J_{out}	$E_{oc}=-32$ mV, $E_{cv}=0$ mV[c]	
					$\bar{\mu}_j^o/\bar{\mu}_j^i$	α_j^i (μeq ml^{-1}) (passive)[d]
Maximum	plasmalemma	8.0	4.3	0.80	1.35	7.2
	tonoplast	14.3	7.3	0.58	0.58	7.3
Minimum	plasmalemma	5.2	2.8	1.21	2.08	4.9
	tonoplast	1.3	0.67	4.11	4.15	0.67

[a] Activity coefficients adopted were: outside solution 0.96; cytoplasm 0.53; vacuole 0.51.
[b] See footnote[a], Table 2.
[c] Transmembrane potential differences. E_{oc}, across plasmalemma; E_{cv}, across tonoplast.
[d] Ion activity which would result from the electrochemical activity ratios if all the fluxes were passive.

vacuole Mg^{2+} concentrations (Table 3). These were converted to activities on the basis of coefficients (Table 3, footnote a) related to the total cation concentration in each compartment (see Macklon, 1975a). From these values, the fluxes given in Table 2, and the transmembrane electrical potentials given by Macklon (1975a) for onion root cortical cells, flux ratios and electrochemical activity ratios were calculated (Table 3). On the basis of both maximum and minimum estimates of vacuolar Mg^{2+} content, the electrochemical activity ratio ($\bar{\mu}_j^o/\bar{\mu}_j^i$) exceeds the flux ratio across the plasmalemma, and is equal to the flux ratio across the tonoplast. Thus, with the Ussing-Teorell equation as the criterion, the Mg^{2+} activity in the cytoplasm is less than would be expected as a result of passive diffusion whilst Mg^{2+} is passively distributed across the tonoplast.

Estimation of ^{28}Mg leakage from the cut ends of the segments, separately from the surface efflux from the tissue, gives a measure of Mg^{2+} transport to the stele during the elution period. The results (Fig. 2) are expressed as μ equivalents per gram fresh weight, on the basis of the specific activity of the loading solution. After rapid loss from the "free space", attributed largely to damaged cells of cortex and stele, transport to the stele was fairly constant for about 4 h and was overwhelmingly in the basipetal direction. Thus, the steady state flux from the proximal end was $0.95 \pm 0.06 \times 10^{-2}$ μeq $g^{-1}h^{-1}$, whilst from the distal end it was only $0.04 \pm 0.02 \times 10^{-2}$ μeq $g^{-1}h^{-1}$ (means of 2 experiments). Mg^{2+} attributed to the "free space" averaged 0.025 ± 0.006 μeq g^{-1} fresh weight at the proximal end and 0.046 ± 0.003 μeq g^{-1} fresh weight at the distal end.

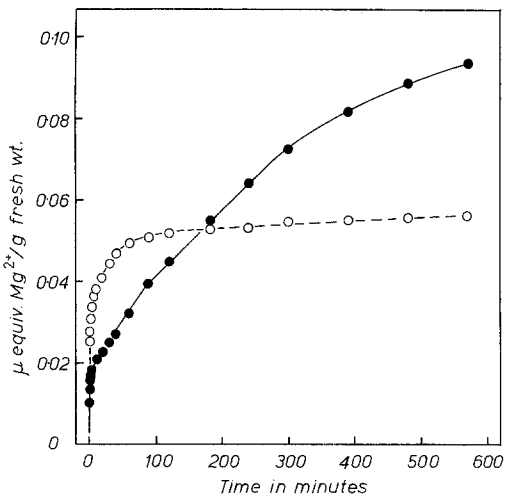

Fig. 2. Example of the time course for the appearance of Mg^{2+} at the distal (o---o) and proximal (●—●) ends of root segments, during elution in unlabelled solution

Discussion

In most respects, magnesium was found to behave like calcium. A component of the efflux curve, with a $t_{\frac{1}{2}}$ (Table 1) intermediate between that associated with the cytoplasm ($t_{\frac{1}{2}}$ about an hour) and that usual for exchange in the water free space ($t_{\frac{1}{2}}$ less than 10 min) was identified with the Donnan free space (DFS). The part played by the DFS in producing a shoulder in the absorption time course curve for Ca^{2+}, and its partial reduction by a 30 min 0° C desorption period in unlabelled solution, has already been discussed (Macklon, 1975b). Similar considerations apply to Mg^{2+}, i.e. the half times of exchange are such that even after an uptake time of less than 30 min, not all the activity in the outer compartments is washed out by a 30 min desorption period, and once all the free space compartments have equilibrated with the specific activity of the loading solution (after about 70 min) the amount of isotope removed per unit weight remains constant (Fig. 1).

Since the Ca^{2+} results were obtained using a solu-

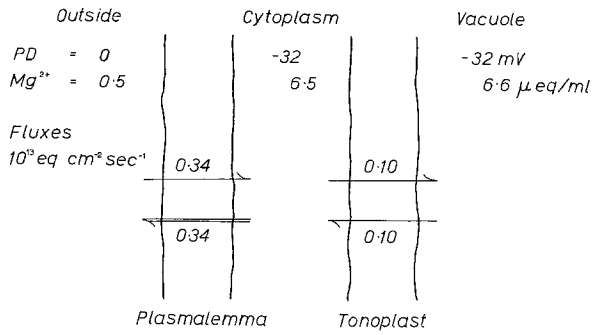

Fig. 3. Flux diagram, summarizing the best estimates of fluxes and compartment concentrations of Mg^{2+} and indicating the location of the proposed ion pump (⇒) and sites of passive diffusion (→)

tion of the same composition as that used in the present work, it is possible now to make an estimate of the total capacity of the DFS in onion root segments. The Ca^{2+} content of the DFS was found to be 1.90 μeq g^{-1} fresh weight, and the Mg^{2+} content of the DFS to be 0.25 μeq g^{-1} (Table 1), giving a total capacity of 2.15 μeq g^{-1}. If the exchange sites are considered to be evenly distributed through the cell wall, which occupies about 9% of the tissue volume, this represents a concentration of non-exchangeable anions equal to 24 μeq ml^{-1}. Thus the DFS content is of the same order as that found for barley leaf slices (3.6 μeq g^{-1}) by Pitman *et al.* (1974) but, because of the much higher proportion of cell wall material in onion roots compared with barley leaf, the apparent concentration of non-exchangeable anions is less than one tenth of the value quoted by Pitman *et al.* (280 μeq ml^{-1}).

In estimating Mg^{2+} fluxes across plasmalemma and tonoplast (Table 2), wide limits were set on the Mg^{2+} content of the vacuole, since no direct measure of this value was available. Hot water extracts of root tissue removed no more than 70% of the total magnesium content, and although turnover of the remaining Mg^{2+} may occur fairly readily (99% of radioactive Mg^{2+} could be removed by extraction with a hot solution containing stable Mg^{2+}), the distribution of this fraction within the cell was not known. However, vacuolar content (Q_v) may be estimated indirectly as that value which is commensurate with the observed net flux of zero, in the same way as has been described for Ca^{2+} (Macklon, 1975b). For Mg^{2+} a vacuolar content of 5.2 μeq g^{-1} was found to yield a flux ratio of one across both plasmalemma and tonoplast.

Whatever value is attributed to Q_v within the wide limits set, it is clear from Table 3 that, under the conditions used here, at least, Mg^{2+} enters the cytoplasm of onion root cortical cells passively, and that the amount in the cytoplasm is limited by an efflux pump at the plasmalemma. Furthermore the value of Q_v adopted does not affect the equivalence between the flux ratio (J_{in}/J_{out}) and electrochemical activity ratio ($\bar{\mu}_j{}^o/\bar{\mu}_j{}^i$) across the tonoplast (Table 3). In this respect Mg^{2+} differs from Ca^{2+}, for which there appears to be an efflux pump at both the plasmalemma and tonoplast (Macklon, 1975b).

The conclusions reached from the compartmental analysis are summarized in Fig. 3, the calculations for which are based on that vacuole content of Mg^{2+} (5.2 μeq g^{-1}) which yields flux ratios of one across each membrane. Vacuole and cytoplasm contents have been converted to concentrations, and fluxes are expressed on a unit area basis, using values for compartment volumes and membrane surface areas reported earlier (Macklon, 1975a). Permeability to Mg^{2+} calculated on the basis of the Goldman model was found, like permeability to Ca^{2+} (Macklon, 1975b), to be quite high ($P_{Mg}=2.6\times10^{-8}$ cm s^{-1}, calculated from influx) at the plasmalemma and much lower at the tonoplast ($P_{Mg}=0.30\times10^{-8}$ cm s^{-1}, calculated from both influx and efflux).

The pattern of Mg^{2+} leakage from the cut ends of root segments was very similar to that for Ca^{2+} (Macklon, 1975b), except that the absolute quantity transported was 75% less, in keeping with the 1:4 ratio of Mg^{2+} to Ca^{2+} in the bathing solution. Again there is a marked polarity of transport, so that extremely little Mg^{2+} appeared at the distal ends of the segments. It is difficult to formulate an explanation of the differential discrimination between Ca^{2+} and Mg^{2+}, which both move almost exclusively in a basipetal direction in the stele, and Na^+ which moves only acropetally (Macklon, 1975a). In an earlier discussion of this problem (Macklon, 1975b) it was considered possible that the findings could be explained if basipetal transport occurred in the xylem and acropetal transport only in the phloem. However, it is generally considered that Mg^{2+}, unlike Ca^{2+}, is mobile in the phloem, and for the explanation to remain tenable, a discrimination by the phloem against Mg^{2+} absorption would seem necessary.

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