The $^{45}Ca^{2+}$ Uptake by *Trichoderma viride* Mycelium. Correlation with Growth and Conidiation

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Abstract. The properties of Ca^{2+} uptake by *Trichoderma viride* were studied using radionuclide $^{45}Ca^{2+}$ in conjunction with the study of effects of agents influencing the Ca^{2+} homeostasis on the $^{45}Ca^{2+}$ uptake, vegetative growth and conidiation. Mycelium of *T. viride* was found to take up $^{45}Ca^{2+}$ in time- and temperature-dependent manner. The $^{45}Ca^{2+}$ uptake could be distinguished from the $^{45}Ca^{2+}$ binding by the insensitivity to washing with EGTA (ethylene glycol-bis(2-amino ethyl ether)-N,N,N',N'-tetraacetic acid)-containing solution. The $^{45}Ca^{2+}$ uptake was only slightly suppressed by the treatment used to de-energize cells. Agents known to influence Ca^{2+} homeostasis in animal and plant cells were also active in perturbing the Ca^{2+} homeostasis in *T. viride*. In this respect, the agents tested had dual (stimulatory or inhibitory) effects on the $^{45}Ca^{2+}$ uptake. No clear correlation among the perturbation of the $^{45}Ca^{2+}$ uptake and the inhibition of growth and conidiation was found for the group of compounds tested. Sr^{2+} and Mg^{2+} inhibited $^{45}Ca^{2+}$ uptake but did not inhibit growth and conidiation. Co^{2+}, Cd^{2+} inhibited both $^{45}Ca^{2+}$ uptake and growth. Other agents tested (Cu^{2+}, Ni^{2+}, La^{3+}, dihydropyridines), which inhibited growth of *T. viride*, induced massive $^{45}Ca^{2+}$ uptake by its mycelium. Ba^{2+} and Mn^{2+} showed a biphasic effect on $^{45}Ca^{2+}$ uptake-inhibition at lower, and stimulation at higher concentrations, but they had only a slight inhibitory effect on the growth or conidiation at higher concentrations. The $^{45}Ca^{2+}$ uptake was influenced by addition of monovalent cations to a small extent only. Na^+ (up to 75 mmol.l^{-1}), less than K^+, slightly suppressed the $^{45}Ca^{2+}$ uptake leaving both growth and conidiation unaffected. Upon depriving the fungus of Ca^{2+} by chelation of extracellular Ca^{2+} (not Mg^{2+} or divalent trace metals) by EGTA, which interfered with Ca^{2+} homeostasis, vegetative growth rate, and starvation-induced conidiation were restricted. These results suggest that the sustained Ca^{2+} influx occurs across the *T. viride* plasma membrane which may be a

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target site for the antifungal action of heavy metal ions, and its perturbation may lead to disturbances in physiological processes including growth and conidiation. The properties of the Ca^{2+} influx in *T. viride* observed substantially differ from those observed in animal cells.

Key words: *Trichoderma viride* growth and conidiation — Effects of Ca^{2+} chelators and Ca^{2+} blockers — EGTA — Nitrendipine — Nifedipine — Divalent cations — $^{45}Ca^{2+}$ uptake.

Introduction

It is generally accepted that animal, plant and bacterial cell growth and other functions require Ca^{2+} ions (for review see Rosen 1982; Hepler and Wayne 1985; Carafoli 1987; Bean 1989). These are necessary for maintaining processes of membrane biogenesis, contractility, transmembrane signalling, and differentiation. The understanding of Ca^{2+} homeostasis in fungal cells is rather scarce (for review see Horák et al. 1984, O'Day 1990), although there are indications that Ca^{2+} ions may play an important role in the morphogenesis and differentiation of fungi, (Reissig and Kinney 1983; Muthukumar and Nickerson 1984; Pitt and Poole 1984; Dicker and Turian 1990; Muthukumar et al. 1991; Robson et al. 1991a,b; Pitt and Barnes 1993), in particular in the growth of hyphal tips (for review see Jackson and Heath 1993). In this paper we describe properties of the influx pathway of Ca^{2+} homeostasis in *T. viride* which help to elucidate the role of the latter in both vegetative growth and differentiation.

Materials and Methods

Trichoderma viride, strain CCM F-534 from the Czechoslovak Collection of Microorganism, T.G.Masaryk University, Brno, Czech Republic, was used.

Cultivation

The fungus was grown in Petri dishes (d = 120 mm) on Czapek Dox agar containing 0.5% (w/v) yeast autolysate at 25 °C. The plates (in duplicates) were inoculated by filter paper disks (5 mm in diameter) soaked in a suspension of conidia in 0.02% Tween 20 (v/v) under red light illumination, and were incubated in the dark at 25 °C. All inspections of plates and measurements of colonies were done under red light in order to prevent the photoconidiation.

Growth

Growth was evaluated by measuring diameters of colonies after 24, 48, and 72 h of cultivation. When effects of inhibitors and/or activators were tested, these were added to the medium before inoculation. When EGTA was used as the tested substance, the agar medium was buffered with 40 mmol.l^{-1} Tris – maleate buffer, pH 6.5. When dimethyl-

sulphoxide was used as the solvent, its final concentration did not exceed 0.5% (v/v) in all samples.

Conidiation

Agar plates without inhibitors were inoculated with 5 mm filter paper disks soaked with a suspension of conidia which were put into the center of another filter paper disk (45, or 70 mm in diameter) located on the agar surface and the plate was incubated in the dark. The larger disk with a growing colony was then transferred onto a new agar plate (containing substances to be tested) and cultivated in the dark for additional 72 h. (When EGTA was used as the tested substance, the agar medium was buffered with 40 mmol.l^{-1} Tris – maleate buffer, pH 6.5.) The measurements of the colony diameter were carried out under the safe red light. At the end of the experiments, conidia were inactivated by formaldehyde vapours (1 h), quantitatively scraped from the plates, suspended in 0.02% Tween 20, homogenized in a Potter Elvejhem homogenizer, filtered through a glass filter (G1) to remove hyphae and hyphal fragments, and the volume of the filtered suspension was adjusted to 20 ml. Conidia were counted using a haemocytometer.

$^{45}Ca^{2+}$ uptake

Suspension of hyphae from submerged cultures were used for these experiments. Two hundred ml of liquid Czapek-Dox medium in 500 ml flasks containing 0.5% yeast autolysate were inoculated by *T. viride* conidia, and the suspension was cultivated on a rotatory shaker (4 Hz) for 20 h at 25 °C. The mycelial suspension was centrifuged at 1500 rpm (500 × *g*) for 10 min, the supernatant was aspirated and the mycelium was washed with Czapek-Dox medium without yeast autolysate. The centrifugation and washing was repeated three times and, finally, the mycelium was suspended in Czapek-Dox medium to a final volume of 70 ml. The mycelial suspension had to show no apparent inhomogeneities. It was kept at ambient temperature and immediately used for experiments. Aliquots of the suspension were incubated with $^{45}Ca^{2+}$ (final concentration 0.55 mmol.l^{-1} specific activity about 1000 cpm/nmol) for 1 h, or the time indicated in the Figures, at 25 °C. Subsequently, 1 ml aliquots were withdrawn and filtered on a membrane filter using a vacuum filtration apparatus and a Millipore nitrocelulose filter (pore diameter 0.65 μm), or Whatman GF/A microfiber filter. The pellet was washed with 2 × 4.5 ml Czapek-Dox medium containing 10 mmol.l^{-1} EGTA, pH 7.0, and was taken for the liquid scintillation counting. Where indicated in the Figure, EGTA was omitted from the washing fluid.

Determination of the mycelium weight

Ten ml aliquots of the suspension vere excessively drained on membrane filter to determine the wet weight, and dried at 90 °C to a constant weight to determine the dry weight.

Chemicals

The chemicals used were from the following sources: Radionuclide $^{45}CaCl_2$ from Radiochemical Centre, Amersham, U.K.; ethylene glycol-bis (2-aminoethylether) – N,N,N',N' – tetraacetic acid (EGTA) and dimethylsulphoxide (DMSO) were from Merck, Darmstadt, Germany; mannitol from Serva, Heidelberg, Germany; FCCP, from Calbiochem, Luzern, Switzerland; $NaVO_3$ from Reachim, Moscow, Russia; tris – (hydroxymethylamino) methane, maleic acid and agar were purchased from Medika, Bratislava, Slovakia. Nifedipine and nitrendipine were obtained from the Institute for Drug Research, Modra,

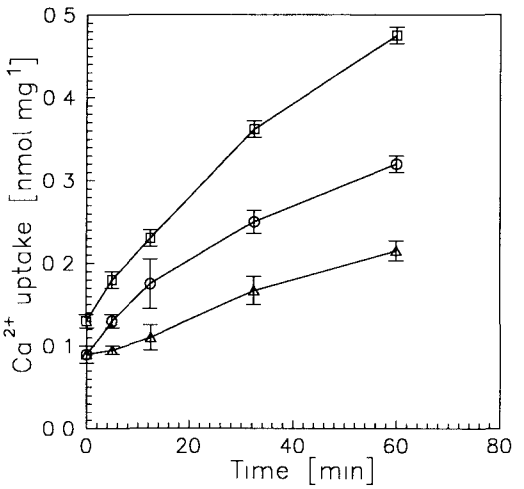

Figure 1. $^{15}Ca^{2+}$ retention in the *T. viride* mycelium in various experimental conditions. $^{15}Ca^{2+}$ (analytical concentration 0.1 mmol l^{-1}) was added to the stock suspension in Czapek-Dox medium kept at 25°C (circles and squares) or at 0°C (triangles) and 1 ml aliquots were withdrawn at the times indicated and the mycelium was separated from the medium by membrane filtration. The mycelium was washed twice with 1.5 ml of either Czapek/Dox medium (squares) (concentration of free Ca^{2+} 0.15 mmol l^{-1}) or by Czapek-Dox medium containing 10 mmol l^{-1} EGTA (concentration of free Ca^{2+} 0.05 μmol l^{-1}) (circles, triangles). The experimental points represent average ± standard error of samples treated in parallel.

Slovakia by courtesy of Dr. Zdeno Mahrla. All other reagents were purchased from Lachema, Brno, Czech Republic.

Results

Properties of the $^{15}Ca^{2+}$ uptake in the T. viride mycelium

Basic observations

Pre-incubation of the mycelium suspended in the Czapek-Dox medium with radioactive $^{15}Ca^{2+}$ (the analytical concentration of Ca^{2+} was 0.55 mmol l^{-1}) revealed that there is a time- and temperature-dependent Ca^{2+} uptake into the mycelium (Fig. 1). The uptake was defined as the $^{15}Ca^{2+}$ activity retained in the mycelium extensively washed (2×4.5 ml) with the Czapek-Dox medium containing 10 mmol l^{-1} EGTA which decreased the extracellular Ca^{2+} concentration in our conditions below 10^{-7} mol l^{-1} (calculated with the BAD program by Brooks and Storey (1992) using our conditions and the contaminant Ca^{2+} concentration 0.15 mmol l^{-1} in the Czapek-Dox medium as determined volumetrically). Values of the

order of 10^{-1} mmol.mg^{-1} dry weight.h^{-1} were measured. When EGTA was omitted from the medium, the activity of $^{45}Ca^{2+}$ retained on filters increased approximately twice (Fig. 1), and probably corresponded to the extracellular binding of $^{45}Ca^{2+}$ to the mycelium. It should be mentioned that to some extent the observed $^{45}Ca^{2+}$ uptake occured also at 0°C, although it was substantially lower as compared with that at 25°C (Fig. 1). Also, the $^{45}Ca^{2+}$ uptake was found to be dependent on the extracellular Ca^{2+} concentration. It was saturatable with Ca^{2+}, and corresponded to the low-affinity calcium uptake with the $K_{M(Ca)}$ in the 10^{-4} mol.l^{-1} range (not shown).

Energetic aspects of $^{45}Ca^{2+}$ uptake

As shown in Fig. 1, the $^{45}Ca^{2+}$ uptake occured to some extent also at 0°C; this raised the question on its dependence on metabolic energy. Treatments known to impair the energetic functions in animal cells were found to modify the "resting" $^{45}Ca^{2+}$ uptake (Fig. 2). The presence of glycolysis inhibitors like monoiodacetate, in combination with the non-metabolizable substrate analogue 2-deoxyglucose, inhibited the $^{45}Ca^{2+}$ uptake only partly even after prolonged incubation with the inhibitors, while the presence of inosine reverted the effect (Fig. 2*A*). Uncoupler of oxidative phosphorylation such as FCCP (Fig. 2*B*), and vanadate, an inhibitor of ATP-dependent transport and ATP-dependent enzymic processes, rather stimulated the uptake (Fig. 2*B*). Therefore, the process of the $^{45}Ca^{2+}$ uptake does not seem to be dependent on metabolic energy.

The mechanism of $^{45}Ca^{2+}$-uptake

In order to obtain the information on the mechanism of $^{45}Ca^{2+}$ uptake we measured its response to both inorganic and organic calcium blockers and to changes in the composition of monovalent cations in the medium.

We tested effects of several inorganic calcium blockers on $^{45}Ca^{2+}$ uptake in concentrations ranging from 10^{-6} to 10^{-2} mol.l^{-1}. Divalent/trivalent cations exerted profound effects on the $^{45}Ca^{2+}$ uptake. These effects were different in their quantitative and qualitative aspects. Three types of effects could be distinguished (Fig. 3*A* – *D*): a) a monophasic inhibition (Mg^{2+}, Sr^{2+}, Co^{2+}, Cd^{2+}) (Fig. 3*A*); b) biphasic effect (Ba^{2+}, Mn^{2+}) (Fig. 3*B*, *D*) and c) monophasic activation (La^{3+}, Cu^{2+}) (Fig. 3*C*). The IC_{50} of ions acting as monophasic inhibitors were in our conditions 0.65 mmol.l^{-1} for Cd^{2+}, 1.1 mmol.l^{-1} for Sr^{2+}, 11 mmol.l^{-1} for Mg^{2+}. The IC_{50} values for other ions could not be derived from the experimental data as the degree of inhibition did not reach 50%. The inhibition of the $^{45}Ca^{2+}$ uptake by lower concentrations of Ba^{2+} and Mn^{2+} became apparent when plotted on a scale similar to those in the Fig. 3*A* (Fig. 3*D*). The value of IC_{50} for Ba^{2+} was 0.14 mmol.l^{-1}. The activation of the $^{45}Ca^{2+}$ uptake by ions of the groups b) and c) occurred at different concentrations of the individual ions (Fig. 3*B*, *C*). The lowest

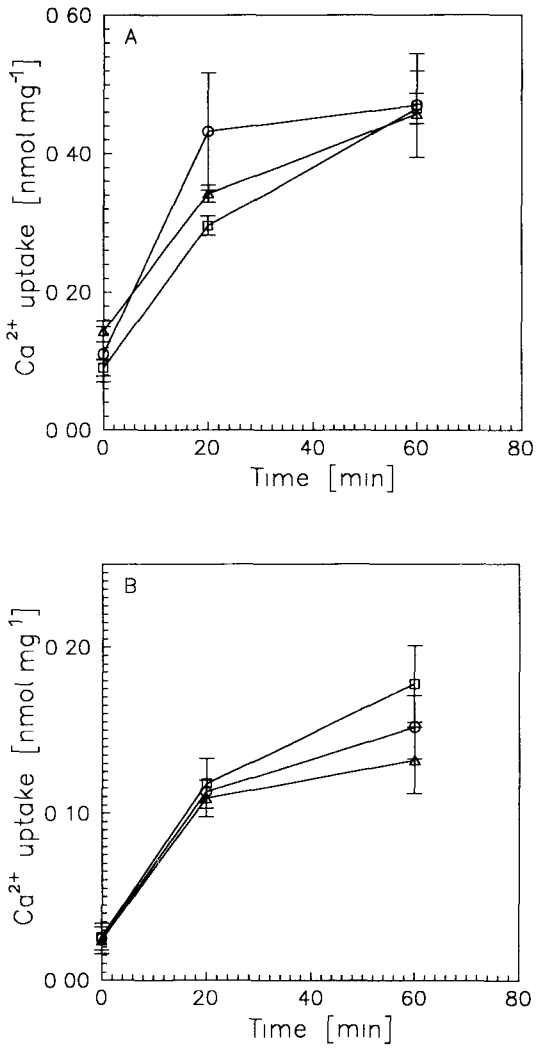

Figure 2. Energetics of the $^{1}Ca^{2+}$ uptake Mycelium was prepared for the measurement of the $^{45}Ca^{2+}$ uptake by washing with modified Czapek-Dox medium without glucose and yeast extract as described in Materials and Methods Aliquots of the suspension were supplemented with the following *A* 20 mmol l^{-1} glucose (triangles) 20 mmol l^{-1} 2-deoxyglucose and 10 mmol l^{-1} iodoacetate (circles) 2-deoxyglucose and iodoacetate as above and 10 mmol l^{-1} inosine (squares) The suspension was incubated at 25 °C for 2 hours and the kinetics of the $^{45}Ca^{2+}$ uptake was measured *B* 20 mmol l^{-1} glucose (triangles) 20 μmol l^{-1} FCCP (circles) 20 μmol l^{-1} FCCP plus 1 mmol l^{-1} $NaVO_3$ (squares) The suspension was incubated at 25 °C for 2 hours and the kinetics of the $^{45}Ca^{2+}$ uptake was measured Results in *A* and *B* are from independent experiments

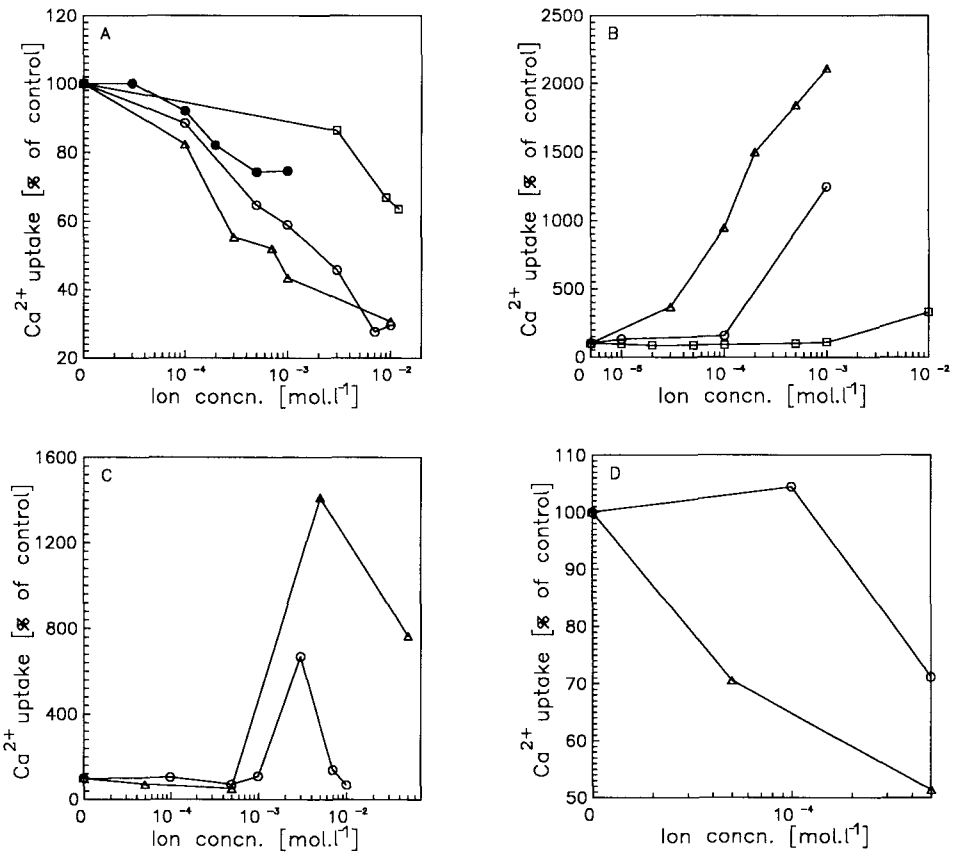

Figure 3. The effect of multivalent ions on the $^{45}Ca^{2+}$ uptake. Aliquots of the suspension were supplemented with concentrated solutions of ion chlorides so that the concentrations of additions were those indicated in the Figure and after 5 minutes, $^{45}Ca^{2+}$ was added (0.5 mmol.l^{-1} final) and the uptake in parallel samples was measured as described in Materials and Methods. The following ions were used: *A*. Mg^{2+} (0.313) (squares); Co^{2+} (0.473) (closed circles); Sr^{2+} (0.421) (open circles); Cd^{2+} (0.744) (open triangles). *B*. Cu^{2+} (0.316) (triangles); La^{3+} (0.194) (circles); Ni^{2+} (0.217) (squares). *C*. Ba^{2+} (0.597) (triangles); Mn^{2+} (0.594) (open circles). In *D*, the values from *C* at lower ion concentrations are plotted in a different scale. Results were corrected for the uptake at zero time and are expressed as % of controls without additions. Absolute values of control samples in nmol.mg^{-1}.h^{-1} are shown in parentheses after the symbols of elements. The individual ions were tested in independent experiments.

one (in the order of 10 μmol.l^{-1}) was that of Cu^{2+}, followed by La^{3+} (in the order 100 μmol.l^{-1}), and Mn^{2+}, Ba^{2+}, and Ni^{2+} (over 1 mmol.l^{-1}). No such inhibitory phase on the dose-effect curves obtained with Mn^{2+} and Ba^{2+}, preceding the acti-

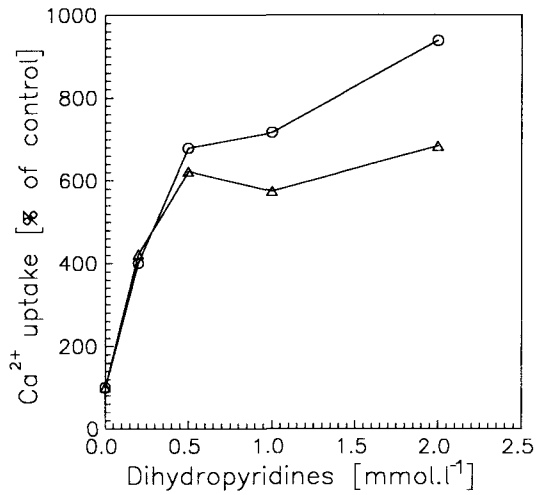

Figure 4. The effect of dihydropyridine calcium blockers on $^{45}Ca^{2+}$ uptake by the *T. viride* mycelium. Aliquots of the suspension were supplemented with concentrated solutions of nifedipin (triangles) or nitrendipin (circles) in DMSO so that the concentrations of additions were those indicated in the Figure; DMSO concentration in all samples was 0.5% (v/v). For all other conditions, see legend to Fig. 3. Absolute values of the uptake in controls were 0.393 nmol.mg^{-1}.h^{-1} for both compounds.

vation phase of the $^{45}Ca^{2+}$ uptake and being small but reproducible (Fig. 3*C*, *D*), was unambiguously expressed with Ni^{2+} (Fig. 3*B*).

Dihydropyridines nifedipine and nitrendipine, known as organic Ca^{2+} blockers, stimulated the $^{45}Ca^{2+}$ uptake, and no biphasic pattern were observed even at the lowest concentrations tested (Fig. 4).

Also the effects on the $^{45}Ca^{2+}$ uptake of other compounds found to inhibit growth (neuroleptic drug chlorprothixene, dyes rhodamin B and thioflavine S, ruthenium red, acridine) were tested. These compounds increased the $^{45}Ca^{2+}$ uptake when used in effective concentrations (0.1–1.0 mmol.l^{-1}) (not shown). Also ouabain, an inhibitor of the Na,K-ATPase, and *p*-chloromercuribenzoate, an inhibitor of HS – groups, increased the $^{45}Ca^{2+}$ uptake at 0.1 mmol.l^{-1}, and 0.01 mmol.l^{-1} (not shown).

In animal cells, the $^{45}Ca^{2+}$ uptake is usually dependent on the composition of the media in respect to monovalent cations, due to the presence of specific transport systems the activity of which depends on the ionic gradients (voltage-dependent Ca channel, Na/Ca antiporter). Therefore, we explored also the effect of monovalent cations. In media containing Tris - salts instead of Na^+ or K^+ salts, the addition of milimolar concentrations of either NaCl or KCl had only marginal effect on

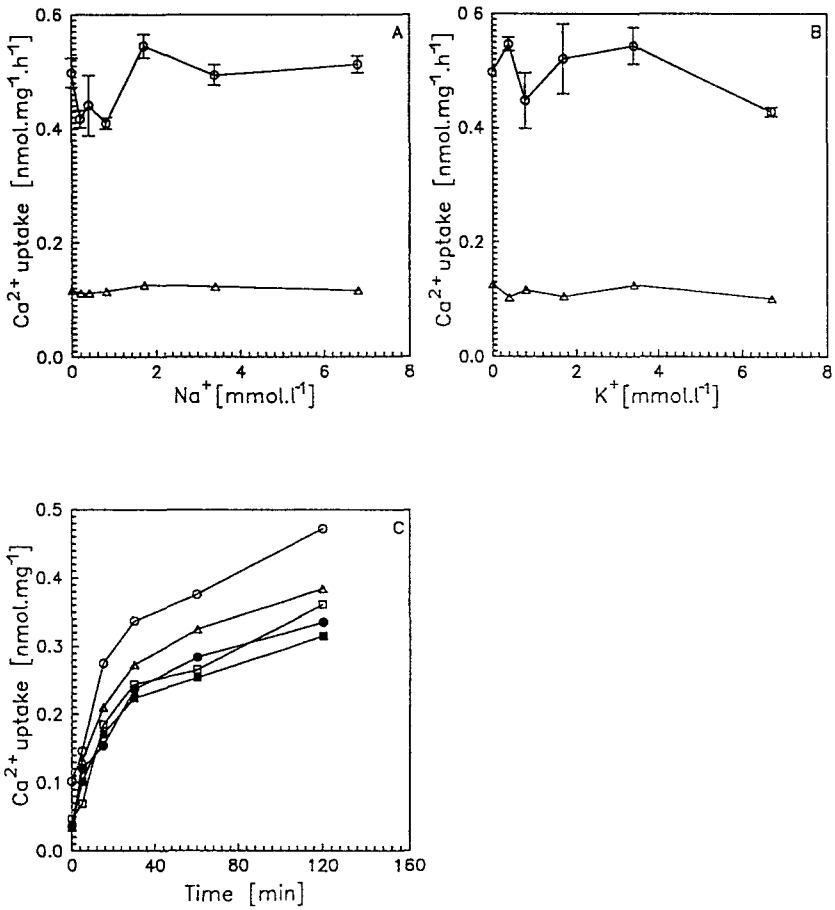

Figure 5. The effect of monovalent cations on $^{45}Ca^{2+}$ uptake by the *T. viride* mycelium. Mycelium was washed in the modified Czapek-Dox medium with both Na^+ and K^+ ions substituted with $Tris^+$ ions. The $^{45}Ca^{2+}$ uptake was measured at 0 (circles) and 60 minutes (triangles) in the presence of the indicated concentrations of NaCl (*A*) or KCl (*B*). In *C*, mycelium was suspended in modified Czapek-Dox media containing 150 mmol.l^{-1} mannitol (open circles), 75 mmol.l^{-1} NaCl (open squares), 75 mmol.l^{-1} KCl (closed circles), or 75 mnmol.l^{-1} choline chloride (closed squares) and the kinetics of the $^{45}Ca^{2+}$ uptake was measured. The control suspension (without additions) was treated in parallel (open triangles).

the $^{45}Ca^{2+}$ uptake (Fig. 5*A*, *B*), and suggested that K^+ may slightly suppress it at higher concentrations. This could be confirmed in independent experiments using higher concentrations of salts (75 mmol.l^{-1}) (Fig. 5*C*). Choline also slightly inhibited the $^{45}Ca^{2+}$ uptake while mannitol added to make up the same osmolarity

stimulated it (Fig. 5*C*). Thus, no K^+-stimulating effect on the $^{45}Ca^{2+}$ uptake was observed in *T. viride* cells.

Perturbation of the Ca^{2+} homeostasis and the growth and conidiation of T. viride

The effects of agents effective in perturbing the Ca^{2+} homeostasis on the growth and conidiation of *T. viride* were studied in order to reveal possible physiological correlates of the Ca^{2+} perturbation. We tested several divalent cations and the Ca^{2+}-specific chelator EGTA as well as dihydropyridines in concentrations effective in influencing Ca^{2+} homeostasis. In general, both growth and conidiation were affected by the agents perturbing the Ca^{2+} homeostasis. The results of these experiments are summarized in Table 1. EGTA in milimolar concentrations significantly inhibited growth and conidiation. Dihydropyridines (nifedipine, nitrendipine) inhibited the growth in the concentration range of 10^{-4} mol/l^{-1} which corresponds to their effect on the $^{45}Ca^{2+}$ uptake. Divalent cations had complex effects on both growth and conidiation.

Of a number of divalent cations tested (Sr^{2+}, Ba^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Cd^{2+}, Mg^{2+}) as well as Cr^{3+} and La^{3+}, at concentrations up to 30 mmol.l^{-1}, only Co^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, Mg^{2+} and La^{3+} significantly inhibited growth of colonies (see Table 1). The inhibitory concentration range of all effective divalent cations was between 1–10 mmol.l^{-1}, with a descending inhibitory potency $Cu^{2+} > Cd^{2+} > Co^{2+} > Ni^{2+} > Mg^{2+}$. La^{3+} ions known as potent Ca^{2+} antagonists inhibited vegetative growth if present at concentration higher than 10 mmol.l^{-1} (Table 1). Other ions did not inhibit growth in the tested concentration range; Mn^{2+}, and Ca^{2+}, Sr^{2+}, and Ba^{2+} significantly (up to 40%) stimulated the vegetative growth of *T. viride* (Table 1). Thus, the divalent cations and La^{3+} which are regarded as inorganic Ca^{2+} antagonists (Kostyuk 1982) exerted complex effects on the vegetative growth, different from effects of organic antagonists.

Effects of divalent ions on the conidia formation were also tested. The effects on the conidiation was also a complex one. Mg^{2+} and Ni^{2+}, but not Cd^{2+} and Cu^{2+}, at concentrations which significantly inhibited the vegetative growth (e.g., 3–7 mmol.l^{-1}), stimulated the formation of conidia in the dark as compared with controls; however, higher concentrations (e.g., 10 mmol.l^{-1}) did not exert such an effect. Rather, they inhibited the conidia formation (Table 1) (Co^{2+} stimulated conidia formation at about 1 mmol.l^{-1} – not shown). These results deserve a separate treatment, and will not be mentioned in detail here. La^{3+} inhibited conidiation in aged colonies similarly as did EGTA in the concentration range between 10 to 30 mmol.l^{-1}, but stimulated conidiation at lower concentrations (not shown). The inhibitory effect of La^{3+} on the starvation-induced conidiation could be reverted by a simultaneous presence of equimolar Ca^{2+} (not shown).

Table 1. Effects of agents influencing Ca^{2+} homeostasis on the growth and conidiation of *Trichoderma viride*

Addition	Concentration mmol l^{-1}	Growth % of control	Conidiation % of control
None	0	100	100
EGTA	5	48	33
	10	16	0
Nisoldipine	0 06	84	n d
	0 60	69	n d
Nifedipine	0 06	92	n d
	0 60	76	n d
Nitrendipine	0 06	90	n d
	0 60	38	n d
Verapamil	0 06	98	n d
	0 60	94	n d
La^{3+}	10	95	n d
	20	0	5 8
Mg^{2+}	5	98	135
	10	95	82 5
Ni^{2+}	5	97	105
	10	38	72 5
Co^{2+}	5	50	58 7
	10	26	8 0
Cd^{2+}	5	34	6 0
	10	0	0
Cu^{2+}	5	26	5 0
	10	0	0
Ca^{2+}	5	125	124
	10	125	143
Sr^{2+}	5	117	111
	10	133	106
Ba^{2+}	5	107	110
	10	140	100
Mn^{2+}	5	145	103
	10	122	34 5

n d not determined The values were obtained in independent experiments performed in duplicate using procedures described in Materials and Methods

Discussion

The presented results describe some basal features of the Ca^{2+} homeostasis in *T viride* and at the same time, show that perturbation of the Ca^{2+} homeostasis influenced both the growth and the conidiation of *T viride* It is feasible to assume that the effects of EGTA reflect a continuous re-cycling of Ca^{2+} across the

cytoplasmic membranes of cells via an inward-transporting carrier(s), and some unknown outward-directed pumping mechanism(s). This notion is supported by the fact that the mycelium could be labelled by $^{45}Ca^{2+}$ in a time- and temperature-dependent manner. Also, the inhibitory effects of several divalent cations (Cd^{2+}, Mg^{2+}, Sr^{2+}) (Fig. 3) on the $^{45}Ca^{2+}$ uptake point out the existence of a carrier catalysing an inwardly directed Ca^{2+} influx inhibited by solute analogues. On the other hand, other divalent cations stimulated the $^{45}Ca^{2+}$ uptake or had biphasic effects depending on their concentrations. The biphasic effects of Ba^{2+}, Ni^{2+}, Mn^{2+} and the activation effects of other cations suggest that these divalent cations and La^{3+} also act on additional site(s) on the membrane with a different, mainly lower, affinity, and open an additional Ca^{2+} influx pathway. Such an explanation has been used for the biphasic effects of heavy metal cations on the vanadate-induced Ca^{2+} uptake by red blood cells (Varečka et al. 1986) based on findings of Lukacovic et al. (1984). Adopting this interpretation one could explain the absence of the inhibitory phase in the effects of Cu^{2+}, Ni^{2+}, La^{3+} and dihydropyridines (Fig. 3 and 4) by supposing that the effect on the second site of action starts at the same or even lower Ca^{2+} concentration than that on the Ca^{2+} carrier, and that both effects overlap. Unlike in red cells where the role of the anion channel is strongly supported (Lukacovic et al.1984), the nature of the second site is unclear and remains to be elucidated.

In our experiments a disparity was observed between the effects of divalent ions on the vegetative growth and/or conidiation, and on the $^{45}Ca^{2+}$ uptake. The closest calcium congeners (Mg^{2+}, Sr^{2+}, Ba^{2+}) and Mn^{2+} did not inhibit growth and conidiation but all of them inhibited the $^{45}Ca^{2+}$ uptake. Only heavy metal cations (Cu^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}) and La^{3+} had effect on the Ca^{2+} homeostasis and simultaneously inhibited the vegetative growth and conidiation. These observations present two discrepancies. The first one is between the (dual) character of the effect on the $^{45}Ca^{2+}$ uptake and the (uniform) effect on growth. This discrepancy could be explained by assuming that both the decrease and the increase of the Ca^{2+} influx has a deleterious effect on the fungal physiology and/or that the Ca^{2+} pathway opened by higher concentrations of pertubing agents may be conductive also to them so that they could be introduced into the cytoplasm together with Ca^{2+}. The second discrepancy concerns the absence of any physiological response and the inhibition of the $^{45}Ca^{2+}$ uptake. The negative effect of Sr^{2+} on the growth and the differentiation could be expected as Sr^{2+} behaves exactly as Ca^{2+} in Ca^{2+}-transporting and -binding systems (Schatzmann 1982), and this predestinates Sr^{2+} for the inhibition of $^{45}Ca^{2+}$ uptake. The effects of the remaining members of this group (Mg^{2+}, Mn^{2+}, Ba^{2+}) might be explained if the main part of the total Ca^{2+} requirements necessary for growth during the period tested (72 hours) is covered from intracellular sources and, at the same time, cells have means how to eliminate the penetrated ions from the cytoplasm. So far, there is no information available

which could support or disprove our explanation.

The inhibitory effects of dihydropyridine Ca^{2+} blockers on the growth of *Trichoderma viride* is similar to that in excitable animal cells (for review see Kostyuk 1982), in *Ceratocystis ulmi* (Muthukumar and Nickerson 1984), in *Penicillium notatum* (Pitt and Poole 1984), in *Neurospora crassa* (Reissig and Kinney 1983) and *Fusarium graminearum* (Robson et al. 1991a,b). We found that the inhibitory effect of these agents on the growth did not parallel the results of direct measurements of Ca^{2+} uptake where stimulation was observed, similarly as shown for some divalent ions (see above). It was shown previously that the dihydropyridine compounds known to be inhibitors or stimulators of the Ca^{2+} uptake in muscle cells, had different effects in the vanadate-induced Ca^{2+} uptake by red blood cells (Stimpel et al. 1984), i. e., the inhibitory and/or the activatory effect of individual dihydropyridines seems to be dependent also on the experimental model used. Summarizing the effects of all calcium antagonists we can state that both the restriction of the Ca^{2+} influx and the overload of cells by Ca^{2+} which was shown to be even lethal in other types of cells (Schanne et al. 1979) may underly the inhibition of *T. viride* growth by calcium antagonists. Using *Saccharomyces cerevisiae* as a model, Kováč (1985) investigated the Ca^{2+} requirement in yeasts. He observed a similar effect of EGTA on the growth as was shown herein. Recently, the possible explanations of the effect of EGTA has been discussed in a review by Youatt (1993). Recently, Gadd and Brunton (1992) have shown that externally added Ca^{2+} was involved in the dimorphism induction of *Ophiostoma ulmi*. Authors described in detail also the properties of the Ca^{2+} uptake. Thus, the study of the Ca^{2+} homeostasis may be useful for explaining also physiological aspects of fungi.

The studies of the Ca^{2+} homeostasis in fungi including the present one have indicated that some aspects of transport processes in fungi are different from those observed in animal cells. For example, all Na^+, K^+, choline$^+$ chlorides suppressed the uptake suggesting that there is no involvement of monovalent cation permeability in the Ca^{2+} influx. The opposite effect of mannitol may rather indicate some role of chlorides in this process. The analysis of these differences could be of value for the understanding of the Ca^{2+} homeostasis in fungi.

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