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UPTAKE AND ACCUMULATION OF Mn^{2+} AND Sr^{2+} IN *SACCHAROMYCES CEREVISIAE*

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Initial uptake of Mn^{2+} and Sr^{2+} in the yeast *Saccharomyces cerevisiae* was studied in order to investigate the selectivity of the divalent cation uptake system and the possible involvement of the plasma-membrane ATPase in this uptake. The initial uptake rates of the two ions were not significantly different. This ruled out a direct role of the plasma-membrane ATPase, since this ATPase is specific for Mn^{2+} compared to Sr^{2+}. After 1 h uptake, Mn^{2+} had accumulated 10-times more than Sr^{2+}. Influx of Mn^{2+} and Sr^{2+} remained unchanged during that time, however. The differences in accumulation level found for Mn^{2+} and Sr^{2+} could be ascribed to a greater efflux of Sr^{2+} as compared with Mn^{2+}. Probably this greater efflux of Sr^{2+} was only apparent, since differential extraction of the yeast cells revealed that Mn^{2+} is more compartmentalised than Sr^{2+}, giving rise to a lower relative cytoplasmic Mn^{2+} concentration.

Introduction

The presence of a transport system for divalent cations in the yeast cell membrane was demonstrated by Rothstein et al. [1]. The uptake was remarkably stimulated when the cells were pretreated with phosphate in the presence of glucose [1,2]. The system was found to be selective for Mg^{2+} and Mn^{2+}, the affinity series being Mg^{2+}, $Co^{2+} > Zn^{2+} > Mn^{2+} > Ni^{2+} > Ca^{2+} > Sr^{2+}$ [1,2]. Fuhrmann et al. [3] found the same sequence for the activation of the plasma-membrane ATPase and suggested that this ATPase was directly involved in the transport of divalent cations. Peters and Borst-Pauwels [4], using a more purified preparation of the plasma-membrane ATPase, found also an activation by Co^{2+} and Mg^{2+}, but no activation by Ca^{2+} and Sr^{2+} up to 50 mM. Moreover, the Mg^{2+}-ATPase was inhibited by Ca^{2+} and Sr^{2+}.

The selectivity of the uptake system was concluded from measurements of uptake of divalent cations over long periods of time, even more than 1 h [1,2]. However, it might well be possible that the initial rates of uptake are not much different, a fact that may readily escape attention [5]. According to Fuhrmann et al. [3] Sr^{2+} is not taken up whereas Ca^{2+} is still accumulated though at a slow rate. Roomans et al. [5], determining initial rates of uptake, found Sr^{2+} uptake rates that were not much different from those of Ca^{2+}. We have studied Mn^{2+} and Sr^{2+} uptake in order to see if the same selectivity is found in the initial uptake of these ions as was found over long periods of time or if there is no selectivity as far as initial rates of uptake are concerned. A preliminary report of this work has been presented elsewhere [6].

Materials and Methods

Yeast cells, *Saccharomyces cerevisiae* strain Delft II, with a low phosphate content were starved overnight under aeration. After starvation, the cells (2%,

Abbreviations: Mes, 2-(*N*-morpholino)ethanesulfonic acid; DEAE-Dextran, diethylaminoethyldextran.

w/v) were washed and incubated for 1 h at 25°C in 45 mM Tris succinate (pH 5.0) provided with 3% glucose (w/v), 10 mM Tris phosphate and 0.1 mM $MgCl_2$. The cells were kept anaerobically by bubbling nitrogen through the suspension. After the incubation period the cells were washed twice and incubated for 20 min in 45 mM Tris succinate (pH 7.0) provided with 3% glucose (w/v) at 25°C, again under anaerobic conditions. Uptake of Mn^{2+} and Sr^{2+} (added to the medium as chloride salts) was studied using ^{89}Sr and ^{54}Mn, respectively, as a tracer with the technique described by Borst-Pauwels et al. [7]. Samples of the yeast suspension were washed with ice-cold 50 mM EDTA (adjusted to pH 8.5 with NaOH), filtered and dried with acetone; the radioactivity was determined by means of an end-window Geiger-Müller tube in the case of ^{89}Sr and a scintillation probe (containing an NaI crystal) in the case of ^{54}Mn. Plasma-membrane ATPase was isolated and purified according to Ref. 4. The Mg^{2+}-ATPase activity was determined by measuring orthophosphate liberation from 2 mM Na_2-ATP (vanadate-free; Boehringer Mannheim) in a 50 mM Tris/50 mM Mes buffer (pH 6.9) containing 1 mM $MgCl_2$ and 250 mM KCl, as described in Ref. 4.

Differential extraction of soluble pools with DEAE-Dextran was performed according to Ref. 8. For this extraction cells of a non-flocculant brewing yeast, *Saccharomyces cerevisiae* strain A 294 (obtained from Whitbread and Co., Ltd., Luton, U.K.) were used. The cells were grown on a medium containing 10 g yeast extract (Difco), 20 g Bactopeptone (Difco), 20 g glucose, 2 g $MgCl_2 \cdot 6\,H_2O$, 39.6 g KH_2PO_4 and 0.4 g $K_2HPO_4 \cdot 3\,H_2O$ per l. The pH was adjusted to 4.5 with HCl. After harvesting in the early exponential phase of growth by centrifugation, the cells were washed twice with 45 mM Tris-succinate buffer (pH 7.0). After 20 min incubation in this buffer, provided with 3% glucose (w/v), the cells were incubated for 1 h with 1 mM of the respective divalent cation. The incubations were performed under anaerobic conditions and at 25°C. Control cells were incubated in the same way without further additions. Immediately after this incubation period the cells were extracted with DEAE-Dextran (Pharmacia, Uppsala, Sweden), for details see under Results. The amounts of Mn and Sr in the different fractions were determined with an atomic absorption spectrophotometer (Beckman-1272), the amount of K was measured with a Zeiss flame spectrophotometer. Electron spin resonance spectra of Mn^{2+} in the Tris-succinate buffer and in aquadest were recorded with a Varian EPR Spectrometer at room temperature.

Results

The uptake of Mn^{2+} and Sr^{2+} during 60 min is shown in Fig. 1. Between approx. 5 and 60 min Mn^{2+} accumulated much more than Sr^{2+}, just as has been found by Rothstein et al. [1]. The uptake within the first 5 min is shown in the inset of Fig. 1. Since the uptake showed deviations from linearity after 30 s the initial rates were calculated using first-order reaction kinetics. Fig. 2 shows the initial rate of Mn^{2+} and Sr^{2+} uptake calculated in this way. The initial uptake rate of the two ions was not significantly different, however.

The low accumulation of Sr^{2+} compared to Mn^{2+} may be explained in at least two ways. Evidence has been presented that the membrane potential can drive the uptake of divalent cations in yeast [5,9]. Since it has been suggested that the plasma-membrane ATPase might act as a proton pump [10], this enzyme may be involved indirectly in the uptake of divalent cations by generating an electrogenic potential across the yeast cell membrane. If this is true, the difference in the amount of Sr^{2+} and Mn^{2+} taken up after prolonged incubation may be due to either an impairment of the plasma-membrane ATPase by the Sr^{2+} accumulated into the cells, or an enhancement of that enzyme by the Mn^{2+} accumulated. By this either the Sr^{2+} influx rate decreases during Sr^{2+} uptake or the Mn^{2+} uptake rate increases during Mn^{2+} uptake. In support to this hypothesis we found that the Mg^{2+}-ATPase activity of the purified membrane preparation was inhibited for already 22% by 1 mM Sr^{2+}, while 1 mM Mn^{2+} enhanced this activity with 36%.

To test this possibility further we examined whether the ^{89}Sr influx rate decreases on incubating the cells with 1 mM Sr^{2+} by comparing the influx of ^{89}Sr initially with that of the isotope after 1 h incubation with 1 mM Sr^{2+}. The results of this experiment are shown in Fig. 3. There was no significant difference between both influx rates. This was also found for ^{54}Mn uptake in the presence of 1 mM Mn^{2+} (Fig. 4). Therefore, changes in the influx rate cannot explain the observed difference in accumulation between the two cations.

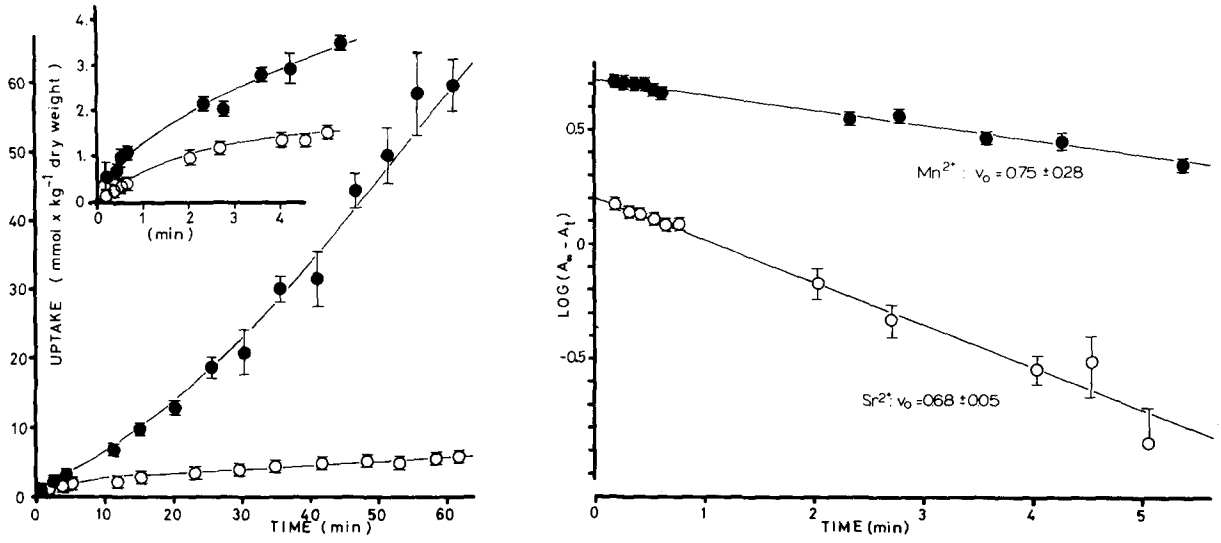

Fig. 1. Accumulation of Mn^{2+} (•) and Sr^{2+} (○) at pH 6.5. The initial concentration was 1 mM. The bars denote the standard error of the mean (n = 3). Inset: Initial uptake of Mn^{2+} (•) and Sr^{2+} (○). The solid line represents the first-order analysis as described in Fig. 2.

Fig. 2. Semi-logarithmic plot of the data of Fig. 1. A_∞ is the amount of divalent cation accumulated in the cells in the steady state, and was estimated by means of a computer fit in such a way that straight lines are obtained in the semi-logarithmic plot. A_t is the amount accumulated at the time indicated. Initial uptake rate (v_0) is expressed as mmol/min per kg dry weight ±S.E. (n = 3).

A second possibility is that Sr^{2+} is extruded via a specific efflux mechanism for which Mn^{2+} has no or only a low affinity. The uptake rate of Sr^{2+} between 30 and 60 min incubation in the experiment of Fig. 3 was 0.053 mmol/kg dry weight per min. Since the initial influx rate of ^{89}Sr after 60 min incubation with 1 mM Sr^{2+} was 0.76 mmol/kg dry weight per min, apparently there must be an efflux of 0.71 mmol Sr^{2+}/kg dry weight per min under these conditions. The experiment of Fig. 4 with Mn^{2+} gives an efflux of 0.53 mmol Mn^{2+}/kg dry weight per min. Cells preloaded for 1 h with carrier free ^{89}Sr and ^{54}Mn also showed an extrusion of the isotope. These extrusions were not affected by the presence of 1 mM of the unlabeled cation in the medium (results not shown). The relative rate of efflux of the divalent cations (i.e., efflux rate divided by total intracellular concentration) was 0.12 and 0.04 for Sr^{2+} and Mn^{2+}, respectively. Therefore, the rate by which Sr^{2+} leaves the cells may be 3-times higher than the efflux rate of Mn^{2+}. A more pronounced efflux of Sr^{2+} compared to Mn^{2+} could easily explain the difference in accumulation found after 1 h incubation.

Since there are indications that Mn^{2+} is accumulated in the vacuole of the yeast cell [11], it is also possible that the different rates of efflux of Mn^{2+} and Sr^{2+} are only apparent and should be ascribed to a preferential accumulation of Mn^{2+} in the vacuoles. Recently, Huber-Wälchli and Wiemken [8] have developed a method for studying localisation of metabolites and enzymes within the yeast cell. In this method first the plasma membrane is rapidly disrupted by a small dose of DEAE-Dextran under isotonic conditions. Vacuolar membranes, in contrast, remain intact and the vacuoles can be extracted in a second step with 60% (w/v) methanol. Since cells of the strain Delft II so far used in this study were resistant against DEAE-Dextran lysis, use was made of another strain for these extractions (see under Materials and Methods). With cells of this strain essentially the same results were found for the uptake of Mn^{2+} and Sr^{2+} (results not shown). Table I shows the results of a typical extraction experiment carried out with cells of this strain. Almost all the Mn^{2+}

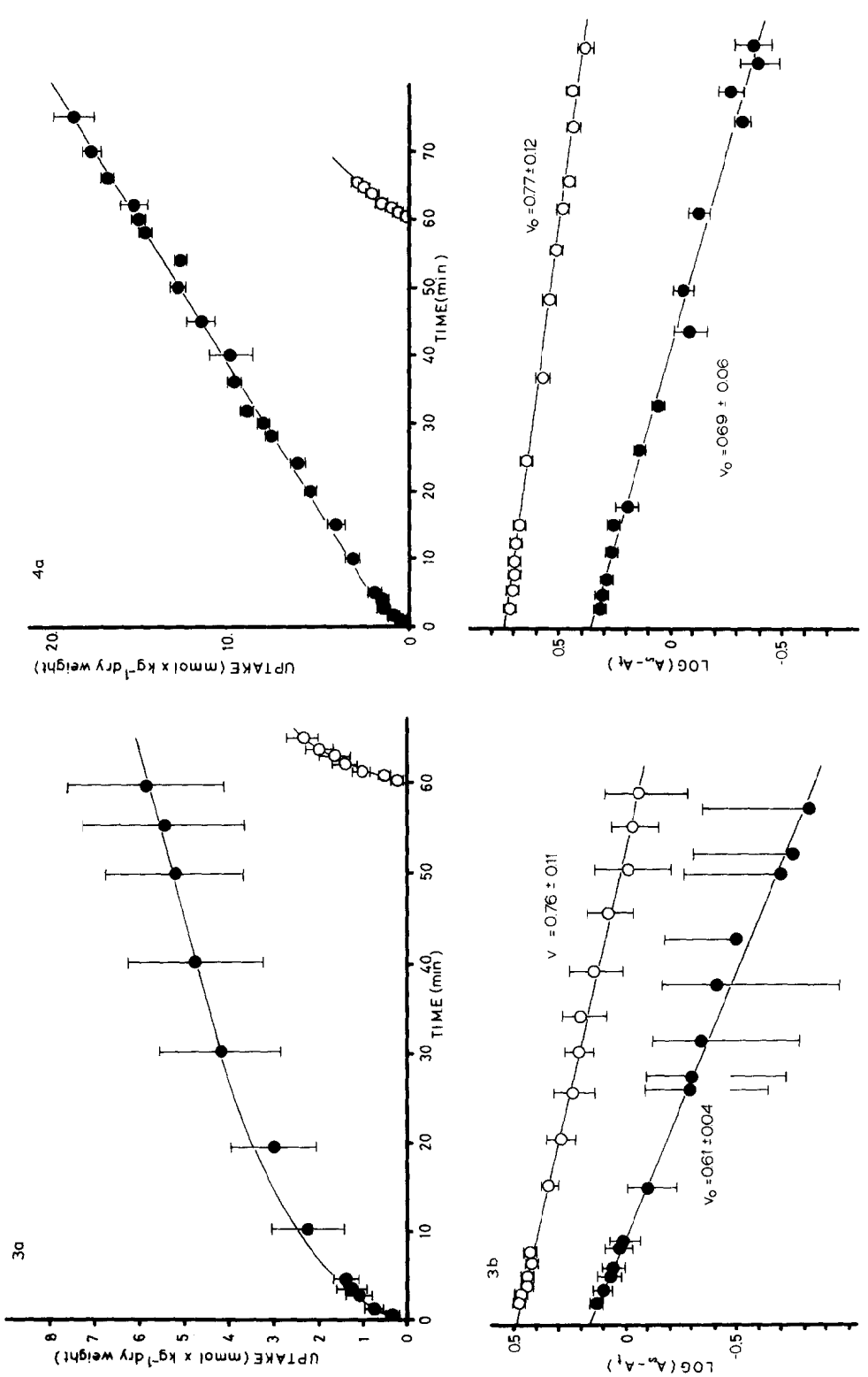

Fig. 3. (a). Accumulation of Sr^{2+} at pH 6.5, the initial concentration was 1 mM. (●) initial uptake and accumulation of ^{89}Sr during the incubation with 1 mM Sr^{2+} ($t = 0$); (○) initial uptake of ^{89}Sr after 1 h incubation with 1 mM Sr^{2+} ($t = 60$ min). In the latter case the cells were centrifuged after 60 min and resuspended in buffer (pH 6.5) with ^{89}Sr and 1 mM $SrCl_2$. Uptake measurements were performed as described in Materials and Methods. The bars denote the standard error of the mean ($n = 3$). (b). Semi-logarithmic plot of the influx of Sr^{2+} at $t = 0$ and $t = 60$ min. For the mean of v_0 see Fig. 2.

Fig. 4. (a). Accumulation of Mn^{2+} at pH 6.5. The initial concentration was 1 mM. (●) initial uptake and accumulation of ^{54}Mn during the incubation with 1 mM Mn^{2+} ($t = 0$); (○) initial uptake of ^{54}Mn after 1 h incubation with 1 mM Mn^{2+} ($t = 60$ min), for details see the legend to Fig. 3. (b). Semi-logarithmic plot of the influx of Mn^{2+} at $t = 0$ and $t = 60$ min, for the meaning of v_0, see Fig. 2.

TABLE I

DISTRIBUTION OF Mn^{2+}, Sr^{2+} AND K^+ IN CELLS OF *SACCHAROMYCES CEREVISIAE* (STRAIN A294)

Cells were loaded as described in Materials and Methods. Washing of the cells and the extractions were carried out at 0–4°C. The following solutions were used: 10 mM Mes/10 mM Tris (pH 6; buffer); 0.7 M sorbitol in buffer (buffered sorbitol); and 60% methanol (v/v) in buffer. The cells (7 mg dry weight per ml) were washed twice with buffer and once with buffered sorbitol. After these washings the cells were resuspended in buffered sorbitol. A small amount of a DEAE-Dextran solution in buffered sorbitol was added under mixing to give a final concentration of 0.3 mg DEAE-Dextran per ml. After 1 min incubation the cells were centrifuged, the remaining cell pellet was washed with buffered sorbitol and immediately centrifuged. Both supernatants were collected (DEAE-Dextran fraction); the cells were then resuspended in 60% methanol and immediately centrifuged. The remaining cell-pellet was washed with buffer and centrifuged again. Both supernatants were collected (60% methanol fraction). The amounts are presented as percentage of the total amount of the two fractions, ±S.D. (n = 3).

		DEAE-Dextran fraction (%)	60% methanol fraction (%)
Mn^{2+}	Mn^{2+}-loaded cells	10 ± 0	90 ± 0
Sr^{2+}	Sr^{2+}-loaded cells	29 ± 1	71 ± 1
K^+	control cells	37 ± 6	63 ± 6
	Mn^{2+}-loaded cells	80 ± 10	20 ± 10
	Sr^{2+}-loaded cells	47 ± 3	53 ± 3

accumulated in the cells was found in the second extraction step, while this was less pronounced for Sr^{2+}. The partition of K^+ differed only slightly from that of Sr^{2+} and was not affected much by loading of the cells with Sr^{2+}. In the presence of Mn^{2+} there seemed to be a shift of the K^+ to the DEAE-Dextran fraction. Since in this strain the volume of the cytosol compared to that of the vacuoles and other organelles is not known, it is difficult to interpret our results in terms of concentration gradients and to compare them with results from X-ray microanalysis [12] or other published results on differential extraction of yeast cells [8,11,13,14].

It has been suggested that Mn^{2+} would give complex formation with succinate [2], one of our buffer components, which may give rise to an underestimation of the concentration of the free divalent cation in the buffer and therefore an underestimation of the initial uptake rate for this ion. Via ESR [15] we have determined the amount of Mn^{2+} that may be bound to succinate or Tris at pH 7. At maximum 35% of the Mn^{2+} appeared to be involved in complex formation.

Discussion

After Rothstein et al. [1] demonstrated in 1958 the energy-dependent uptake of divalent cations in yeast it has generally been accepted that this uptake is selective for Mg^{2+} and Mn^{2+} compared to Ca^{2+} and Sr^{2+}. A similar affinity series for the divalent cation activation of the isolated plasma-membrane ATPase as for the uptake in whole cells, found during prolonged incubation, has led to the suggestion by Fuhrmann et al. [3] that the ATPase is directly involved in the divalent cation transport in whole cells. The initial uptake rates do not differ significantly, however. This means that the uptake is not selective for these divalent cations and that the different accumulation over longer periods of time cannot be interpreted in terms of selective uptake of these cations. This rules out a direct role of the plasma-membrane ATPase in this transport since this ATPase is highly specific for Mg^{2+} and Mn^{2+} compared to Ca^{2+} and Sr^{2+} [3,4].

Interference of the buffer components with Sr^{2+} can be neglected [15,16] while that for Mn^{2+} is small. Corrections in the rate of Mn^{2+} influx for this decrease in the free Mn^{2+} concentration are at maximum 35%. Although this value is an overestimation, since the uptake of divalent cations shows saturation kinetics, a low selectivity for Mn^{2+} cannot be excluded.

The difference between the net uptake rate of the two ions found after 1 h and the influx rate at this time points to the involvement of an efflux mechanism for these ions. Similarly, efflux of Ca^{2+} from yeast cells has already been demonstrated by Boutry et al. [9], in contrast to earlier findings for Mg^{2+}, Mn^{2+} [1] and Zn^{2+} [18–20]. Ca^{2+}-efflux mechanisms have been demonstrated in animal cells [21], in bacteria for example *Streptococcus faecalis* [22], *Myobacterium phlei* [23], *Escherichia coli* [24] and in the fungus *Neurospora crassa* [25]. These efflux systems are supposed to give the cell a good opportunity to regulate the level of free Ca^{2+} in the cytoplasm [26].

The greater efficiency by which Sr^{2+} is extruded, compared to Mn^{2+}, is most likely the cause for the divalent cation selectivity that is found during prolonged incubation. However, the more rapid efflux of Sr^{2+} may be only apparent. The concentration of free divalent cation in the cytosol may be regulated by storage in organelles like the vacuole, as has been suggested by Okorokov et al. [11], or by binding to cell components. Our results with the differential extraction procedure suggest that Mn^{2+} is compartmentalised to a higher extent than Sr^{2+}. This could explain the smaller relative efflux rate of Mn^{2+} compared to Sr^{2+}, because then the free Mn^{2+} concentration in the cytosol is decreased due to a higher compartmentalisation.

Our results for Mn^{2+} and K^+ in this differential extraction procedure are in good agreement with those of the group of Okorokov [11,13,14] with *Saccharomyces carlsbergensis*. In this yeast K^+ accumulates predominantly in the vacuoles of cells in the mid-exponential growth phase and on Mg^{2+} and Mn^{2+} accumulation into these cells a K^+ efflux from the vacuoles was found [14]. The uneven distribution of K^+ and the divalent cations was assumed to be created by special transport systems localised in the vacuolar membrane. If this supposed transport system is highly selective for Mn^{2+} compared to Sr^{2+} this would indeed lead to more accumulation of Mn^{2+} in the vacuoles. Selectivity for uptake in yeast vacuoles has in fact been found for basic amino acids [8,27] and purines [28].

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