Demonstration of high-affinity Mn\textsuperscript{2+} uptake in Saccharomyces cerevisiae: specificity and kinetics

Geoffrey M. Gadd and Oliver S. Laurence

Author for correspondence: Geoffrey M. Gadd. Tel: +44 1382 344266. Fax: +44 1382 322318. e-mail: g.m.gadd@dundee.ac.uk

The existence of multiple transport systems for Mn\textsuperscript{2+} in Saccharomyces cerevisiae has been demonstrated in this study. Mn\textsuperscript{2+} (supplied as MnCl\textsubscript{2}) was accumulated by S. cerevisiae at all Mn\textsuperscript{2+} concentrations examined (25 nM–1 mM) but a log-log plot of uptake rates and total amounts accumulated revealed the existence of at least two Mn\textsuperscript{2+}-concentration-dependent transport systems. Over a low Mn\textsuperscript{2+} concentration range (25–1000 nM), high-affinity Mn\textsuperscript{2+} uptake occurred with a \( K_m \) value of 0.3 \( \mu \text{M} \), while transformation of kinetic data obtained over the concentration range 5–200 \( \mu \text{M} \) revealed another system with a \( K_m \) of 62 \( \mu \text{M} \). Meaningful kinetic analyses were not possible at higher Mn\textsuperscript{2+} concentrations because of toxicity: only about 30% of cells remained viable after 30 min incubation with 1000 \( \mu \text{M} \) MnCl\textsubscript{2}. Release of K\textsuperscript{+} accompanied Mn\textsuperscript{2+} accumulation and this increased with increasing Mn\textsuperscript{2+} concentration. However, even in non-toxic Mn\textsuperscript{2+} concentrations, the ratio of Mn\textsuperscript{2+} uptake to K\textsuperscript{+} release greatly exceeded electroneutral stoichiometric exchange. In 50 \( \mu \text{M} \) MnCl\textsubscript{2}, the ratio was 1:123 and this increased to 1:2670 in 1000 \( \mu \text{M} \) MnCl\textsubscript{2}, a toxic concentration. External Mg\textsuperscript{2+} was found to decrease Mn\textsuperscript{2+} accumulation at all concentrations examined, but to differing extents. Over the low Mn\textsuperscript{2+} concentration range (5–200 \( \mu \text{M} \)), Mg\textsuperscript{2+} competitively inhibited Mn\textsuperscript{2+} uptake with a half-maximal inhibitory concentration, \( K_i \), of 5.5 \( \mu \text{M} \) Mg\textsuperscript{2+}. However, even in the presence of a 50-fold excess of Mg\textsuperscript{2+}, inhibition of Mn\textsuperscript{2+} uptake was of the order of 72% and it appears that the cellular requirement for Mn\textsuperscript{2+} could be maintained even in the presence of such a large excess of Mg\textsuperscript{2+}. Over the high Mn\textsuperscript{2+} concentration range (5–200 \( \mu \text{M} \)), the \( K_i \) for Mg\textsuperscript{2+} was 25.2 \( \mu \text{M} \). At low Mn\textsuperscript{2+} concentrations, Zn\textsuperscript{2+} and Co\textsuperscript{2+}, but not Cd\textsuperscript{2+}, inhibited Mn\textsuperscript{2+} uptake, which indicated that the high-affinity Mn\textsuperscript{2+} uptake system was of low specificity, while at higher Mn\textsuperscript{2+} concentrations, where the lower-affinity Mn\textsuperscript{2+} transport system operated, inhibition was less marked. However, competition studies with potentially toxic metal cations were complicated due to toxic effects, particularly noticeable at 50 \( \mu \text{M} \) Co\textsuperscript{2+} and Cd\textsuperscript{2+}.

**Keywords:** Mn\textsuperscript{2+}, Mg\textsuperscript{2+}, Saccharomyces cerevisiae, transport kinetics, divalent cations

**INTRODUCTION**

Manganese is probably an essential metal for all micro-organisms and has been implicated in the regulation of growth and metabolism in fungi (Garraway & Evans, 1984; Kubicek & Rohr, 1986; Hockertz et al., 1987a; Pilz et al., 1991; Auling, 1994) as well as in bacteria (Auling, 1983; Archibald, 1986; Plonzig & Auling, 1987), mainly due to its influence on several key enzymes (Hughes, 1990; Kendrick et al., 1992; Frausto da Silva & Williams, 1993). Mn\textsuperscript{2+} has also been proposed as an important eukaryotic cell regulator (Williams, 1982) and, in fungi, it appears to be preferentially located in the vacuole, where it may be bound to polyphosphates (Okorokov et al., 1980, 1985; Lichko et al., 1980; Okorokov, 1985).

Although some divalent cations, e.g. Mg\textsuperscript{2+}, Ca\textsuperscript{2+}, may enter fungal cells via a monovalent cation transport...
system (Borst-Pauwels, 1981; Jones & Gadd, 1990), this is probably of little or no importance in terms of cellular requirements, particularly since external K⁺ will outcompete under most conditions of growth. More generally, divalent cations appear to enter into cells as a result of the electrochemical gradient (ΔÆH⁺) generated by the activity of the plasma membrane H⁺-ATPase (Borst-Pauwels, 1981; Sanders, 1990; Jones & Gadd, 1990; Gadd, 1993), although there is some evidence that an electrochemical K⁺ gradient (ΔÆK⁺) may also be important (Okorokov, 1985; Ramos et al., 1985). While the existence of a Ca⁺⁺-ATPase has been proposed in fungi (Miller et al., 1990), there is no evidence of a comparable mechanism for other essential divalent cations, although divalent cation efflux mechanisms have frequently been proposed (Nieuwenhuis et al., 1981; Okorokov, 1985; Jones & Gadd, 1990).

The extent to which Mg⁺⁺ and Mn⁺⁺ may substitute for each other in fungal metabolism has long been a topic of speculation (Garraway & Evans, 1984) with considerable technical difficulties in attempting to elucidate such roles. Mn⁺⁺/Mg⁺⁺ antagonism has been noted in reciprocal substitution in Mn⁺⁺/Mg⁺⁺-activated proteins as well as in Mn-induced mutagenicity in yeasts due to Mn⁺⁺/Mg⁺⁺ antagonism in DNA metabolism (Auling, 1994). However, if Mn⁺⁺ is essential and specific for certain functions, then specific transport systems should exist for its accumulation regardless of the external ionic composition. Under most environmental and cultural conditions, external Mg⁺⁺ concentrations will be higher than Mn⁺⁺ concentrations and if Mn⁺⁺ transport was blocked by Mg⁺⁺, then deleterious limitation would result. Specific transport systems would enable the accumulation of essential metal cations regardless of the external cationic composition, which may include potentially competing cations.

Many studies on divalent metal cation transport in fungi have been carried out with potentially toxic metals, often essential in low concentrations, like Cu, Zn and Mn, in order to understand mechanisms of accumulation, intracellular storage and relationship with tolerance (see Gadd, 1993). However, kinetic analyses are frequently complicated by the symptoms induced by toxic metals, which can include membrane disruption, and storage in intracellular organelles like the vacuole (Gadd & White, 1989). This may be particularly evident for metals such as Zn⁺⁺ and Mn⁺⁺ which are of relatively low toxicity to fungi (compared to, e.g. Cu⁺⁺, Cd⁺⁺), meaning that toxicity-related work necessitates the use of high non-physiological concentrations. Gadd & White (1989) have concluded that for many potentially toxic metals, the magnitude of affinity constants for transport is largely dependent on the metal concentration range used, with apparent low-affinity systems being detected at high concentrations. Although not enough work has been carried out at low micronutrient metal concentrations, high-affinity metal transport systems have been demonstrated in fungi, predominantly yeasts. A specific transport system for Mn⁺⁺ has been demonstrated in Candida utilis with a Kₘ of 164 μM (Parkin & Ross, 1986), with Saccharomyces cerevisiae able to transport Zn⁺⁺ with a Kₘ of 37 μM (White & Gadd, 1987a). It is surprising that there is little detailed information on Mn⁺⁺ transport in S. cerevisiae since described high-affinity uptake systems for other cations appear less discriminatory against potential competing divalent cations in comparison to other yeasts, e.g. C. utilis (Ross, 1993). This property could affect the ability of S. cerevisiae to obtain adequate trace amounts of Mn⁺⁺ in the presence of competing cations. In addition, a depth of knowledge exists on intracellular Mn⁺⁺ storage in Saccharomyces spp. (Okorokov et al., 1980; Nieuwenhuis et al., 1981; Okorokov, 1985; Kühne et al., 1988).

The objective of this work was therefore to characterize Mn⁺⁺ transport in S. cerevisiae over a range of external concentrations to find out whether this organism possesses multiple transport systems of differing affinities, and to determine their sensitivity to potentially competing divalent cations, particularly Mg⁺⁺.

**METHODS**

**Organism and culture conditions.** Saccharomyces cerevisiae X2180-1B was routinely maintained on MYGP agar of composition (g l⁻¹): malt extract (Lab M), 30; yeast extract (Difco), 3.0; bacteriological peptone (Oxoid), 5.0; d-glucose, 10.0; agar (Lab M no. 2), 15; and incubated at 25 °C. For experimental purposes, cultures were grown at 25 °C on an orbital shaker (100 r.p.m.) in a medium comprising (g l⁻¹): KH₂PO₄, 2.72; K₂HPO₄, 3H₂O, 5.22; (NH₄)₂SO₄, 2.0; MgSO₄. 7H₂O, 0.12; FeSO₄. 7H₂O, 0.022; ZnSO₄. 7H₂O, 0.004; MnSO₄. 4H₂O, 0.004; CuSO₄. 5H₂O, 0.0004; d-glucose, 2.00; yeast extract (Difco), 1.0. Starter cultures were prepared by loop inoculation of the liquid medium from MYGP agar and grown as above for 24-48 h. Experimental cultures were inoculated to an initial OD₅₅₀ of approximately 0.1. Cells were counted using a modified Fuchs–Rosenthal haemocytometer after appropriate dilution with distilled water.

**Mn⁺⁺ uptake by S. cerevisiae.** Late-exponential phase (18 h) cells were harvested by centrifugation (1200 g, 10 min) and washed three times with and finally resuspended in 5 mMPIPES buffer, adjusted to pH 6.5 using solid tetramethylammonium hydroxide, to a density of approximately 2 × 10⁶ cells ml⁻¹. For experiments, cell suspensions were derived from this by dilution in PIPES buffer to a density of 5 × 10⁶ cells ml⁻¹ and contained d-glucose at a final concentration of 50 mM. The suspension was then equilibrated with stirring at 25 °C for 15 min. Mn⁺⁺ uptake was initiated by the addition of MnCl₂. 4H₂O to the required concentration, with ⁸⁸Mn (Amersham International) added as a tracer to a final activity of 37–925 kBq ml⁻¹. Where required, MgCl₂. 6H₂O, CoCl₂. 6H₂O, CdCl₂ or ZnCl₂ at the desired concentration was added to the cell suspension with the MnCl₂. 4H₂O and ⁸⁸Mn. At intervals, 1 ml samples were removed and harvested by filtration through Whatman cellulose nitrate filters (0.45 μm, 25 mm diameter). Preliminary experiments were carried out with samples taken every 2 min during the first 10 min. This established the linear nature of initial uptake. The filters and cells were washed five times with 2.5 ml ice-cold 10 mM MnCl₂. 4H₂O to remove bound ⁸⁸Mn. Filters were then placed in scintillation fluid (Ecoscint A, National Diagnostics) and radioactivity was measured using a Packard Tri-carb 4000 series scintillation counter.

**Measurement of extracellular K⁺.** K⁺ concentrations in solutions were measured using a Corning K⁺-specific electrode
Mn$^{2+}$ accumulation by Saccharomyces cerevisiae

**RESULTS**

**Mn$^{2+}$ accumulation in the absence of competing ions.**

Mn$^{2+}$ was accumulated by *S. cerevisiae* at all the concentrations examined. At low concentrations (25–1000 nM MnCl$_2$), both the initial rate and the final amounts of Mn$^{2+}$ accumulated by the cells increased with increasing concentrations of MnCl$_2$ up to 1000 nM (Fig. 1). When these data were transformed by Lineweaver–Burk analysis and the resultant straight line was fitted according to the least squares method, this transformation gave a $V_{\text{max}}$ value of 3.4 pmol Mn$^{2+}$ min$^{-1}$ per 5 $\times$ 10$^7$ cells and a $K_m$ value of 0.3 $\mu$M MnCl$_2$ (correlation coefficient of fitted line = 0.9543).

Uptake of Mn$^{2+}$ from higher concentrations of MnCl$_2$ (5–200 $\mu$M) (Fig. 1) differed from that at lower concentrations (25–1000 nM) in that the 'uptake profile' was different. The accumulation of Mn$^{2+}$ at the higher concentrations followed a linear pattern over the time-course of the experiment (30 min), whereas at lower concentrations the maximal rate of accumulation occurred within the first 10 min with a gradual decrease in rate after this time. Transformation of data obtained at MnCl$_2$ concentrations of 5–200 $\mu$M (Fig. 1) gave a $V_{\text{max}}$ value of 57.6 pmol Mn$^{2+}$ min$^{-1}$ per 5 $\times$ 10$^7$ cells and a $K_m$ value of 62 $\mu$M MnCl$_2$ (correlation coefficient of line = 0.9949).

At higher concentrations of MnCl$_2$ (600–1000 $\mu$M), Lineweaver–Burk transformation gave negative values of $K_m$ and $V_{\text{max}}$. This was despite the line of best fit having a correlation coefficient of 0.9830. On testing the viability of cells exposed to these higher concentrations, it was found that only 29.6±2.8% of the cells exposed to 1000 $\mu$M MnCl$_2$ remained viable after 30 min (Table 1).

**Induction of K$^+$ release by MnCl$_2$.**

The amount of K$^+$ released from cells, as measured using a K$^+$-specific electrode, decreased with increasing MnCl$_2$ concentration. The rate of K$^+$ release increased as the concentration of MnCl$_2$ increased (Fig. 2); however, the ratio of Mn$^{2+}$ uptake to K$^+$ release was far in excess of a 1:2 stoichiometric electroneutral exchange. Even in the presence of 50 $\mu$M MnCl$_2$ (a non-toxic level), the ratio of Mn$^{2+}$ uptake to K$^+$ release was 1:123 while in the presence of 1000 $\mu$M MnCl$_2$ the ratio increased to 1:2670 (Table 2).

**Mn$^{2+}$ accumulation in the presence of Mg$^{2+}$.**

The effect of MgCl$_2$ on the accumulation of Mn$^{2+}$ by *S. cerevisiae* was investigated at low (25–1000 nM), high (5–200 $\mu$M) and potentially toxic (1 mM) concentrations of MnCl$_2$. Mg$^{2+}$ was found to reduce Mn$^{2+}$ accumulation at all concentrations examined, but to differing extents. Uptake of Mn$^{2+}$ from 100 nM MnCl$_2$ in the presence of a 10-fold molar excess of Mg$^{2+}$ led to a 54.5% reduction in the amount of Mn$^{2+}$ accumulated after 30 min, while a 50-fold molar excess led to a 71.5% reduction. Similar results were obtained for uptake of Mn$^{2+}$ from 1 $\mu$M MnCl$_2$, where the presence of equimolar MgCl$_2$ resulted in a
Table 1. Viability of *S. cerevisiae* X2180-1B after exposure to Mn²⁺

Cells were suspended in 5 mM PIPES buffer, pH 6.5, to a density of 5 x 10⁶ cells ml⁻¹ and incubated with different concentrations of MnCl₂·4H₂O in the presence of 50 mM glucose for 30 min. Values shown are ± SEM.

<table>
<thead>
<tr>
<th>MnCl₂ concn (μM)</th>
<th>10⁻⁵ × c.f.u. ml⁻¹</th>
<th>Viability (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.26 ± 0.47</td>
<td>100.0 ± 6.35</td>
</tr>
<tr>
<td>400</td>
<td>5.33 ± 0.35</td>
<td>101.3 ± 4.72</td>
</tr>
<tr>
<td>600</td>
<td>4.63 ± 0.23</td>
<td>88.1 ± 2.79</td>
</tr>
<tr>
<td>800</td>
<td>4.60 ± 0.10</td>
<td>87.4 ± 1.34</td>
</tr>
<tr>
<td>1000</td>
<td>1.56 ± 0.20</td>
<td>29.6 ± 2.80</td>
</tr>
</tbody>
</table>

Table 2. Ratios of the initial rate of Mn²⁺ uptake to the initial rate of K⁺ released by *S. cerevisiae* X2180-1B in the presence of different concentrations of MnCl₂

Experimental conditions were as for Fig. 1. Rates of K⁺ release were calculated from traces obtained using a K⁺-specific electrode (White & Gadd, 1987b). Initial rates of Mn²⁺ uptake were calculated as described in Methods. Typical results are shown from one of three experiments.

<table>
<thead>
<tr>
<th>Conc of MnCl₂ (μM)</th>
<th>Ratio of Mn²⁺ uptake:K⁺ release</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>1:123</td>
</tr>
<tr>
<td>100</td>
<td>1:99</td>
</tr>
<tr>
<td>200</td>
<td>1:317</td>
</tr>
<tr>
<td>400</td>
<td>1:1289</td>
</tr>
<tr>
<td>600</td>
<td>1:1175</td>
</tr>
<tr>
<td>1000</td>
<td>1:2670</td>
</tr>
</tbody>
</table>

Fig. 2. Rates of Mn²⁺ uptake (O) and K⁺ release (•) from *S. cerevisiae* X2180-1B at varying Mn²⁺ concentrations. Mn²⁺ uptake rates were derived using ⁵²Mn as described previously; rates of K⁺ release were calculated from traces produced using a K⁺-specific electrode as described by White & Gadd (1987a,b).

58.7% reduction while a 5-fold molar excess of MgCl₂ resulted in an 85.1% reduction in the amount of Mn²⁺ accumulated after 30 min.

Transformation of the initial rates of Mn²⁺ uptake (according to Lineweaver–Burk) suggested that the inhibition caused by 1 μM MgCl₂ was competitive, the Kₘ value being 18.3 μM MnCl₂ as compared to 0.3 μM for uninhibited Mn²⁺ accumulation, while the Vₘₐₓ values were 6.0 and 3.4 pmol Mn²⁺ min⁻¹ per 5 x 10⁶ cells, respectively (Table 3). In competitive inhibition, while the Kₘ is increased the Vₘₐₓ should remain unchanged. The effects of Mg²⁺ over the low Mn²⁺ concentration range therefore do not readily fit usual models for competitive or non-competitive inhibition (where the Kₘ remains approximately constant while the Vₘₐₓ decreases). In the presence of 5 μM MgCl₂, the Kₘ was also increased over the control, being 51 μM, and the Vₘₐₓ value increased to 9.6 pmol Mn²⁺ min⁻¹ per 5 x 10⁶ cells (Table 3). A secondary plot of the slope of the lines obtained in the primary double reciprocal plots against concentration of MgCl₂ indicated linear inhibition with a half-maximal concentration, Kᵢ, of 5.5 μM MgCl₂ (correlation coefficient of line = 0.9491).

Inhibition of Mn²⁺ uptake by MgCl₂ also occurred at the higher MnCl₂ concentrations examined. Uptake of Mn²⁺ from 5 μM MnCl₂ in the presence of equimolar MgCl₂ resulted in a 55.5% inhibition of uptake over 30 min as compared to control values, while at 100 μM MnCl₂ equimolar MgCl₂ inhibited Mn²⁺ uptake by 78.4%. The presence of a 10- and 20-fold molar excess of MgCl₂ on Mn²⁺ accumulation from 5 μM MnCl₂ resulted in an inhibition of uptake by 90.4 and 91.1%, respectively.

Thus, it appeared that inhibition of Mn²⁺ uptake from 5 μM MnCl₂ by Mg²⁺ was not increased significantly by increasing the concentration of MgCl₂ above 50 μM. Transformation of the data obtained revealed that in the presence of 5 μM MgCl₂, Mg²⁺ inhibition was of a competitive nature since the Kₘ value was increased while the Vₘₐₓ remained approximately the same in the presence of 5 μM MgCl₂. The new Kₘ and Vₘₐₓ values were
Table 3. Summary of the kinetic constants for Mn\(^{2+}\) uptake by S. cerevisiae

<table>
<thead>
<tr>
<th>Concentration range of MnCl(_2) used (nM)</th>
<th>Concentration of MgCl(_2) (µM)</th>
<th>(K_m) (µM)</th>
<th>(V_{max}) (pmol Mn(^{2+}) min(^{-1}) per 5 x 10(^6) cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-1000</td>
<td>0</td>
<td>0.3</td>
<td>3.4</td>
</tr>
<tr>
<td>5-200</td>
<td>0</td>
<td>62.0</td>
<td>57.6</td>
</tr>
<tr>
<td>25-1000</td>
<td>1</td>
<td>18.3</td>
<td>6.0</td>
</tr>
<tr>
<td>25-1000</td>
<td>5</td>
<td>5.1</td>
<td>9.6</td>
</tr>
<tr>
<td>5-200</td>
<td>5</td>
<td>106.5</td>
<td>47.9</td>
</tr>
<tr>
<td>5-200</td>
<td>50</td>
<td>283.4</td>
<td>46.7</td>
</tr>
<tr>
<td>5-200</td>
<td>100</td>
<td>108.4</td>
<td>13.5</td>
</tr>
</tbody>
</table>

Table 4. Cellular Mg content of S. cerevisiae X2180-18 after exposure to differing MnCl\(_2\):MgCl\(_2\) ratios

Cells were incubated in 5 mM PIPES buffer, pH 6.5, at 25 °C in the presence of 50 mM glucose for 30 min prior to harvesting and analysis. Intracellular Mg is expressed as nmol Mg per 5 x 10\(^6\) cells ± SEM (three replicates).

<table>
<thead>
<tr>
<th>MgCl(_2) concn (µM)</th>
<th>Intracellular Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>MnCl(_2) (50 µM)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>17.2 ± 0.4</td>
</tr>
<tr>
<td>5</td>
<td>16.8 ± 0.3</td>
</tr>
<tr>
<td>50</td>
<td>18.0 ± 0.4</td>
</tr>
<tr>
<td>100</td>
<td>21.1 ± 0.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MnCl(_2) concn (µM)</th>
<th>Intracellular Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgCl(_2) (50 µM)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>17.3 ± 0.5</td>
</tr>
<tr>
<td>5</td>
<td>23.2 ± 0.3</td>
</tr>
<tr>
<td>50</td>
<td>18.0 ± 0.5</td>
</tr>
<tr>
<td>100</td>
<td>17.0 ± 0.2</td>
</tr>
</tbody>
</table>

106.5 µM MnCl\(_2\) and 47.9 pmol Mn\(^{2+}\) min\(^{-1}\) per 5 x 10\(^6\) cells, respectively (correlation coefficient of line = 0.9631), as compared to 62.0 µM MnCl\(_2\) and 57.6 pmol Mn\(^{2+}\) min\(^{-1}\) per 5 x 10\(^6\) cells for control cells (Table 3). In the presence of 50 and 100 µM MgCl\(_2\), the \(K_m\) values for Mn\(^{2+}\) transport were 283.4 µM and 108.4 µM MnCl\(_2\), respectively, with corresponding \(V_{max}\) values of 46.7 and 13.5 pmol Mn\(^{2+}\) min\(^{-1}\) per 5 x 10\(^6\) cells (correlation coefficients for these lines were 0.9948 and 0.9950, respectively; Table 3). This suggests that in the presence of 50 µM MgCl\(_2\), the competition observed was also competitive, while in the presence of 100 µM MgCl\(_2\), competition was of a non-competitive nature since the \(K_m\) value was increased only slightly while the \(V_{max}\) was reduced fivefold. A secondary plot of the slopes obtained in the primary double reciprocal plot indicated linear inhibition with a \(K_i\) value of 25.2 µM MgCl\(_2\) (correlation coefficient of line = 0.9747).

Table 4 shows the intracellular levels of Mg after 30 min exposure to various MgCl\(_2\):MnCl\(_2\) ratios. In the presence of 50 µM MnCl\(_2\), as the extracellular MgCl\(_2\) level was increased so the internal level of Mg slightly increased. However in the presence of 50 µM MgCl\(_2\), the addition of 5 µM Mn\(^{2+}\) led to an increase in the Mg content of 25.4% of the control value. The intracellular level of Mg decreased towards the control value as the concentration of MnCl\(_2\) was increased. The effect of 0, 5, 50 and 100 µM MgCl\(_2\) on Mn\(^{2+}\) accumulation from 1 mM MnCl\(_2\) was also examined (results not shown). It was found that neither the initial rates nor the total amount of Mn\(^{2+}\) accumulated were dependent on MgCl\(_2\), probably because of the toxic effects of MnCl\(_2\) at this concentration.

Mn\(^{2+}\) accumulation in the presence of Cd\(^{2+}\), Co\(^{2+}\) and Zn\(^{2+}\)

The accumulation of Mn\(^{2+}\) from various concentrations of MnCl\(_2\) in the presence of 5 and 50 µM CdCl\(_2\), CoCl\(_2\) and ZnCl\(_2\) was examined. At the lowest Mn\(^{2+}\) concentration (100 nM), 5 µM Cd\(^{2+}\) did not affect the initial rate of uptake but caused an increase in the total amount of Mn\(^{2+}\) accumulated (Fig. 3a). The presence of 50 µM Cd\(^{2+}\) caused a slight decrease in the total amount of Mn\(^{2+}\) accumulated as compared to control levels. Co\(^{2+}\) and Zn\(^{2+}\) both markedly reduced the initial rate and final amount of Mn\(^{2+}\) accumulated, Zn\(^{2+}\) to a greater extent than Co\(^{2+}\) (Fig. 3a). The effects of Cd\(^{2+}\), Co\(^{2+}\) and Zn\(^{2+}\) were most marked in the presence of 100 µM MnCl\(_2\), where all the ions either increased or had no inhibitory effect on rate of Mn\(^{2+}\) uptake (Fig. 3b). The increase in Mn\(^{2+}\) accumulation caused by Co\(^{2+}\) was concentration-dependent, 5 µM CoCl\(_2\) having no effect while 50 µM CoCl\(_2\) caused an increase both in the rate and total amount of Mn\(^{2+}\) accumulated. Interestingly, the presence of 5 µM ZnCl\(_2\) stimulated Mn\(^{2+}\) uptake while 50 µM ZnCl\(_2\) decreased the total amount of Mn\(^{2+}\) accumulated (Fig. 3b).

DISCUSSION

In *Saccharomyces* spp., it is known that Mn is preferentially located in the vacuole, which is believed to function in the regulation of cytosolic Mn\(^{2+}\) homeostasis (Lichko et al., 1980). The vacuolar membrane of *Saccharomyces* spp. possesses a Mn\(^{2+}/H^+\) antiport system for such accumulation into the vacuole, which has an additional role in the storage and detoxification of other metal ions, including Ca\(^{2+}\), Cs\(^{+}\), Li\(^+\), Co\(^{2+}\) and Zn\(^{2+}\) (Eilam et al., 1985; White & Gadd, 1986, 1987a; Cornelius & Nakashima, 1987;...
Fig. 3. Uptake of Mn$^{2+}$ by S. cerevisiae X2180-1B from (a) 100 nM and (b) 100 μM MnCl$_2$·4H$_2$O in the presence of: (i) 0 (○), 5 (□), 50 (■) μM CdCl$_2$; (ii) 0 (○), 5 (■), 50 (△) μM CoCl$_2$; and (iii) 0 (○), 5 (▼), 50 (▼) μM ZnCl$_2$. Cells were suspended in 5 mM PIPES buffer, pH 6.5, containing 50 mM D-glucose to a density of 5 x 10$^6$ ml$^{-1}$. Values shown are means of three replicates; SEM values were smaller than the symbol dimensions in most cases.

Perkins & Gadd, 1993; Gadd, 1993; Ross, 1993). In this study, 1 ml cell suspension (density of 5 x 10$^6$ cells ml$^{-1}$) took up 9.35 x 10$^{-9}$ nmol Mn$^{2+}$ during 30 min incubation in 100 nM MnCl$_2$. If the cell volume, as measured by a Coulter analysis (Meikle et al., 1988), is assumed to be 47 fl, this gives an intracellular concentration of Mn$^{2+}$ of approximately 398 μM. If it is assumed that all the cellular Mn$^{2+}$ is in an unbound ionic form, this represents an uphill transport concentration gradient of approximately 397:1 (inside: outside). However, it is unlikely that all the Mn$^{2+}$ is present in an osmotically free form and Okorokov et al. (1975, 1980) have estimated that 78% of the total Mn$^{2+}$ taken up by yeast was bound to cellular components, with several reports demonstrating sequestration by vacuolar polyphosphate contributing significantly to the bound pool (Okorokov et al., 1980; Borst-Pauwels, 1981; Kihn et al., 1988; Gadd, 1986).

Mn$^{2+}$ accumulation from low (5–1000 nM) MnCl$_2$ concentrations was shown to operate via a high-affinity system having a $K_m$ value of 0.3 μM and a $V_{max}$ of 3.4 pmol Mn$^{2+}$ min$^{-1}$ per 5 x 10$^6$ cells. The specific uptake of Mn$^{2+}$ by the yeast C. utilis has a $K_m$ value of 16.4 nM Mn$^{2+}$ (Parkin & Ross, 1986). Comparable affinity constants for other metal transport systems in yeasts are 0.36 μM (Lawford et al., 1980) and 1.3 μM (Failla et al., 1976) for Zn$^{2+}$ in C. utilis,
3.7 μM for Zn2+ uptake by S. cerevisiae (White & Gadd, 1987a), 3.1 μM for Cu2+ in C. utilis (Parkin & Ross, 1985) and between 1.1 and 4.4 μM for Cu2+ in S. cerevisiae (De Rome & Gadd, 1987; Lin & Kosman, 1990). The Mn2+ system operating in C. utilis appeared highly specific, being unaffected by a 100-fold molar excess of Mg2+, Zn2+, Ca2+, Cd2+, Ni2+ and Cu2+. Uptake was, however, inhibited 30–40% by a 1000-fold molar excess of Mg2+, Zn2+, Ca2+, Cd2+, Ni2+ and Cu2+ (Parkin & Ross, 1986). In the high-affinity Mn2+ system shown here for S. cerevisiae, a similar specificity was not seen. The large increase in the Km value for Mn2+ transport at the lower Mn2+ concentrations in the presence of 1 μM MgCl2 with only a slight increase in Vmax tends to indicate competitive inhibition of this high-affinity system. However, even in the presence of a 50-fold molar excess of Mg2+, inhibition was only of the order of 71.5%. It appears in S. cerevisiae, therefore, that the cellular requirement for Mn2+ could be maintained, even in the presence of a large excess of Mg2+. The reduction in Km at higher Mg2+ concentrations (although the Km is still higher than the Km for Mn2+ transport only), together with a slight increase in Vmax, could be due either to the Mg2+ accumulated affecting the electrochemical proton gradient and/or the surface potential, both of which would decrease the driving force available for Mn2+ accumulation (Borst-Pauwels & Severens, 1984), or, alternatively, the Mg2+ could be competing for the transport system in a non-concentration-dependent manner. In the filamentous fungus Aspergillus niger, a specific high-affinity Mn2+ transport system (Km = 3 μM) has been detected at submicromolar concentrations of Mn2+ which functioned independently of the transport of Mg2+ and Ca2+ but was preferentially inhibited by Zn2+, Cu2+ and Cd2+ (Hockertz et al., 1987b; Auling, 1994). In Penicillium notatum, Mn2+ uptake from nM concentrations occurred by a system with an apparent Km of 4.4 nM which is insensitive to Mg2+, Zn2+, Ni2+, Cu2+ or Co2+ at 1000-fold excess but competitively inhibited by Cd2+ (Starling & Ross, 1990; Ross, 1993). Another uptake system in this organism, with a Km of approximately 22 μM, operated at 10–100 μM Mn2+ and was competitively inhibited by a fivefold excess of the competing cations (Ross, 1993). In this present study, Co2+ and Zn2+ were found to inhibit Mn2+ uptake from low Mn2+ concentrations and to stimulate Mn2+ uptake from high Mn2+ concentrations.

Uptake of Mn2+ from the higher (5–200 μM) concentrations of MnCl2 examined had a Km value of 62 μM and a Vmax of 57.6 pmol Mn2+ min−1 per 5 x 106 cells. This compares with a Km value of approximately 65 μM for the non-specific Mn2+ uptake system of C. utilis (Parkin & Ross, 1985) and a Km and Vmax of 860 μM and 0.51 pmol Mn2+ min−1 per 109 cells, respectively, for S. cerevisiae 6175/11a (Bianchi et al., 1981a,b) and a Km value of approximately 100 μM for S. cerevisiae 431 (Norris & Kelly, 1977). The presence of 5, 50 and 100 μM MgCl2 increased the Km values to 106 μM, 284 μM and 108 μM, respectively. The Ki value for inhibition by MgCl2 was 25.2 μM. This contrasts with the higher Ki values recorded for inhibition of Co2+ uptake below approximately 0.5 mM CoSO4 of 125 μM MgSO4 and a Ki value for Cd2+ uptake below 0.5 mM CdSO4 of 14 mM MgSO4 (Norris & Kelly, 1977). The slight increase in Km and fivefold decrease in Vmax in the presence of 100 μM Mg2+ is possibly indicative of non-competitive inhibition of Mn2+ transport. In the presence of 5 and 50 μM MgCl2, a concentration-dependent increase in the Km was observed with only a slight change in Vmax, indicating competitive inhibition.

Uptake of Mn2+ from the highest concentrations of MnCl2 examined (400–1000 μM) gave negative Km and Vmax values. Viability studies indicated that toxic effects of Mn2+ were likely to have been responsible for this apparent deviation from Michaelis–Menten kinetics (White & Gadd, 1987a, b). These toxic effects are probably responsible for the anomalous uptake rates obtained from 1000 μM MnCl2 in the presence of 5, 50 and 100 μM MgCl2. The connection between K+ release and toxicity was clear and after 30 min exposure to 1 mM MnCl2, plate counts on MYGP medium showed a 71.4% kill. The ratio of Mn2+ taken up to K+ released was far in excess of 1:2 (see Gadd, 1986), even at the non-toxic level of 100 μM MnCl2. The mechanism by which the rate of K+ efflux from metabolizing yeast is controlled is poorly understood (Borst-Pauwels, 1981, 1988) and the effect of divalent cation uptake on this is unlikely to involve a simple 1:2 ratio (Ross, 1993). Okorokov et al. (1983a, b) also found intense K+ release on initiating Mn2+ uptake in Saccharomyces carlsbergensis using high concentrations (3 mM) of MnSO4 and measuring the rate of uptake in a non-defined medium (meaning that the amount of free Mn2+ available for uptake was unknown). Okorokov et al. (1983a, b) proposed that the K+ efflux observed was used to drive the synthesis of high molecular mass polyphosphates which could be involved in sequestration of Mn2+ in the vacuole.

It has been shown that in the μM range the presence of Mn2+ can have an effect on internal Mg2+ concentrations. This would imply that the two ions are interacting in some way and that at these concentrations a more general divalent cation transport system may be operating (see Borst-Pauwels, 1981; Jones & Gadd, 1990; Gadd, 1993; Ross, 1993). The differing effects of Cd2+, Co2+ and Zn2+ on Mn2+ uptake from various concentrations of MnCl2 may imply that different transport processes operate at different concentrations. At the lowest concentrations examined, all the competing metals except Cd2+ inhibited Mn2+ uptake, which indicates that the high-affinity Mn2+ system of S. cerevisiae is of low specificity regarding other divalent metal cations, while at the higher concentrations of MnCl2, where a lower affinity Mn2+ transport system is more likely to operate, inhibition was less marked. Cd2+ and Co2+ at 50 μM even stimulated uptake at higher MnCl2 concentrations as compared to control levels. This could be due to these ions affecting the electrochemical gradient positively, providing a greater driving force for the accumulation of Mn2+, although it is more likely that the increased level of Mn2+ accumulation observed may be due to toxic interactions of these metal ions with the cells. Part of the population of yeast cells may lose its
viability during incubation of the cells with the metal ions while the remainder remain viable (Gadd & Mowll, 1983; Belde et al., 1988). This loss of viability may be due to a permeabilization of the cell membrane by the metal ions, thus exposing more binding surfaces. This would also explain the increase in both the rate and amount of Mn²⁺ accumulated from low Mn²⁺ concentrations in the presence of 5 μM Cd²⁺, which is toxic (Kessels et al., 1985; Belde et al., 1988).

In summary, it appears that S. cerevisiae possesses a high-affinity Mn²⁺ transport system, functional at low Mn²⁺ concentrations, which is of low specificity, being inhibited by Mg²⁺, Co²⁺, Zn²⁺ and Cd²⁺ to varying extents. At higher concentrations of MnCl₂, a transport system of lower affinity is available to the cell. Despite the Mn²⁺ transport system of S. cerevisiae being relatively nonspecific, transport of Mn²⁺ could still occur, even when there was an excess of competing divalent cations such as Mg²⁺. This is important since micro-organisms must be able to acquire essential ‘trace’ divalent cations, even when there is an excess of other divalent cations present in their external environment.

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