Accumulation of Cadmium and Cobalt by *Saccharomyces cerevisiae*

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(Received 13 December 1976)

SUMMARY

Saccharomyces cerevisiae accumulated Co^{2+} and Cd^{2+} by two processes: the first, which was metabolism-independent, was presumably cation binding to the cell surface; this was followed by metabolism-dependent, progressive uptake of relatively large amounts of the cations. Two K^+ ions were released for each Co^{2+} ion taken up in freshly prepared cell suspensions whereas extensive loss of cell K^+ followed the uptake of Cd^{2+} . Co^{2+} and Cd^{2+} appeared to be accumulated via a general cation uptake system, with limited specificity related to the ionic radii of the cations.

INTRODUCTION

During the last decade, the study of metal accumulation by various organisms has been facilitated by the use of isotopes and atomic absorption spectrophotometry for the selective measurement of elements. During the same period, increased concern has developed over the ecological effects of toxic metal pollutants and particularly over the release of cadmium into the environment (Nilsson, 1970; Nordberg, 1974). The absence of cadmium from a useful review of the effects of heavy metals on fungal cells (Ross, 1975) reflects the paucity of information on interactions of micro-organisms and this potentially hazardous metal.

Metal accumulation by micro-organisms generally comprises two phases: a rapid binding of cations to negatively-charged groups on the cell surface, and progressive, usually metabolism-dependent, intracellular cation uptake. Both phases of cation accumulation by yeasts have been described in studies primarily concerned with essentially non-toxic cations (Rothstein & Hayes, 1956; Rothstein *et al.*, 1958; Fuhrmann & Rothstein, 1968; Ponta & Broda, 1970; Failla, Benedict & Weinberg, 1976).

Energy-dependent uptake of several divalent cations by *Saccharomyces cerevisiae* has been described with an affinity series of Mg , Co , Zn > Mn > Ni > Ca > Sr (Rothstein *et al.*, 1958; Fuhrmann & Rothstein, 1968), and a similar affinity series for divalent cation activation of yeast plasma membrane ATPase has indicated that the enzyme activity may be related to cation transport in whole cells (Fuhrmann, Wehrli & Boehm, 1974). In contrast to this apparently general cation uptake system, Zn^{2+} uptake by *Candida utilis* was effected by a specific, energy-dependent pathway, unaffected by several other cations but inhibited by Cd^{2+} (Failla *et al.*, 1976).

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The accumulation of Cd^{2+} by *S. cerevisiae* is described in this paper and compared with the accumulation of Co^{2+} , for which energy-dependent uptake by *S. cerevisiae* has been described previously (Fuhrmann & Rothstein, 1968).

METHODS

Organism and culture conditions. *Saccharomyces cerevisiae* (NCYC431) was maintained at 4 °C on 2% (w/v) nutrient agar (Oxoid) containing 0.1% (w/v) yeast extract (Oxoid) and 1% (w/v) glucose. For metal cation accumulation experiments, the organism was grown at 30 °C at pH 6.8 in medium containing (g l^{-1}): KH_2PO_4 , 2.72; K_2HPO_4 , 5.22; $(\text{NH}_4)_2\text{SO}_4$, 1.98; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.12; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.0022; D-glucose, 10.0; and yeast extract, 1.0. Flasks (2 l) containing 400 ml medium were shaken at 200 rev. min^{-1} on an orbital shaker. Growth was followed turbidimetrically, using an EEL colorimeter (green filter, peak transmission 540 nm), and by culture dry weight measurements, after centrifuging 4 ml samples, washing with 4 ml distilled water and drying the cell pellets at 105 °C for 18 h.

Preparation of cell suspensions. Cultures were harvested during exponential growth (culture dry wt 0.75 to 1.25 mg ml^{-1}), washed twice by resuspension in distilled water, and resuspended in 5 mM-piperazine-*N,N'*-bis(2-ethanesulphonic acid) (PIPES; Good *et al.*, 1966) adjusted to pH 6.5 with tetramethylammonium hydroxide. This buffer has negligible metal-chelating properties. Suspensions were adjusted by dilution with PIPES buffer to approximately 0.4 mg dry wt ml^{-1} by reference to a previously determined extinction-cell dry weight correlation for suspensions of yeast in buffer.

Metal cation accumulation assay. Yeast suspensions (48.5 ml) were shaken in 250 ml Erlenmeyer flasks at 200 rev. min^{-1} in an orbital shaker at 25 °C. Distilled water (0.5 ml) or, where required, 1 M-glucose (0.5 ml) was added 10 min before addition of 1 ml of a metal salt solution (50 $\times$ required final concentration). The effect of carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), an uncoupler of oxidative phosphorylation, on metal cation accumulation was determined after addition of the inhibitor (dissolved in 10 mM-NaOH) to a cell suspension 5 min before glucose addition. Samples (2 ml) were taken at intervals after adding the metal salt and filtered through 0.6 μm pore size, 25 mm diam. membrane filters (Sartorius). Filters were washed immediately with 5 ml PIPES buffer, then transferred to 5 ml centrifuge tubes.

Metal analyses. To each centrifuge tube containing a filter and cells, 0.2 ml 6 M- HNO_3 was added, and the tubes were placed in a boiling water bath for 30 min to release cell-associated metal ions. Samples were made up to 4 ml with distilled water, centrifuged to remove any undigested material, and the supernatant fluids were analysed for the appropriate elements by atomic absorption spectrophotometry using a Rank-Hilger Atomspek H1170 and the standard procedures prescribed in the instrument's manual and data book (Rank-Hilger, Margate, Kent). Quantities of accumulated cations (e.g. cell Co^{2+}) and residual cations (e.g. cell Mg^{2+}) are expressed throughout this paper as nmol (mg dry wt equiv.) $^{-1}$, and the actual cell dry wt ml^{-1} in the suspension is indicated for each experiment.

RESULTS

Nature of Co^{2+} and Cd^{2+} accumulation

Small amounts of Co^{2+} or Cd^{2+} were bound instantaneously on addition of the metal sulphates to *S. cerevisiae*. This apparent surface binding was independent of temperature (5 to 25 °C), the availability of an energy-yielding substrate or the presence of an energy-

Fig. 1. Co^{2+} and Cd^{2+} accumulation by *S. cerevisiae* ($0.41 \text{ mg dry wt ml}^{-1}$) from (a) 0.2 mM-CoSO_4 and (b) 0.2 mM-CdSO_4 : at 5°C , in the absence ($\blacktriangle$) or presence (Δ) of glucose; at 25°C , in the absence ($\square$) or presence ($\circ$) of glucose, or in the presence of glucose and 0.1 mM-CCCP ($\bullet$).

coupling inhibitor (Fig. 1). The surface-binding capacity of *S. cerevisiae* for divalent cations was relatively low by comparison, on an equal cell dry weight basis, with binding capacities of a range of algae, bacteria and filamentous fungi (Norris, 1976). The quantities of surface-bound Co^{2+} and Cd^{2+} were exceeded by the amounts of each cation subsequently accumulated at 25°C in the presence of glucose by a process of progressive cation uptake which was energy-dependent and depressed by low temperature, CCCP or the absence of glucose (Fig. 1). 2-Deoxy-D-glucose is accumulated and phosphorylated but is not an energy source for *S. cerevisiae* (Van Steveninck, 1968). Addition of 2-deoxy-D-glucose instead of glucose in the present experiments did not support cation uptake. Optimum temperatures for uptake of Co^{2+} and Cd^{2+} were between 25 and 30°C . A longer period of pre-incubation of the cells in buffer before adding glucose and cation resulted in lower rates of uptake. The initial rate of Co^{2+} uptake was reduced by 35% when glucose and CoSO_4 additions were delayed until 6 h after preparation of the cell suspension while viability of the cells did not fall during the pre-incubation period (data not shown).

The cell Mg^{2+} concentration was not affected by the accumulation of Co^{2+} and Cd^{2+} (Fig. 2). The release of $74 \text{ nmol K}^+ (\text{mg dry wt})^{-1}$ during uptake of $36 \text{ nmol Co}^{2+} (\text{mg dry wt})^{-1}$ (Fig. 2a) confirmed the previously described efflux of two K^+ ions for each Co^{2+} ion accumulated by yeast (Fuhrmann & Rothstein, 1968). However, the lack of such exchange by K^+ -deficient cells, and during the later stages of prolonged Co^{2+} uptake (continuing for over 3 h after CoSO_4 addition), showed that K^+ loss is not always a feature of Co^{2+} accumulation (Norris, 1976). Addition of Cd^{2+} resulted in a rapid release of approximately 66% of the cell K^+ (Fig. 2b); but no simple stoichiometric relationship of Cd^{2+}

Fig. 2. Effect of Co^{2+} and Cd^{2+} accumulation on the Mg^{2+} and K^+ concentrations in *S. cerevisiae* at 25 °C: (a) cells (0.39 mg dry wt ml⁻¹) received glucose and 0.2 mM- CoSO_4 ; (b) cells (0.42 mg dry wt ml⁻¹) received glucose and 0.2 mM- CdSO_4 . Co^{2+} (○), Cd^{2+} (●), Mg^{2+} (□), K^+ (△).

Fig. 3. Effect of Ca^{2+} on Cd^{2+} accumulation and subsequent K^+ release by *S. cerevisiae* (0.38 mg dry wt ml⁻¹) at 25 °C after glucose addition: Cd^{2+} accumulation from 0.2 mM- CdSO_4 (○) or from 0.2 mM- CdSO_4 plus 0.2 mM- CaSO_4 (●); K^+ release with CdSO_4 (△) or with CdSO_4 plus CaSO_4 (▲).

Table 1. Effect of various metal salts on the cell K^+ concentration of *S. cerevisiae*

Metal salts (all sulphates except $HgCl_2$, $Pb(NO_3)_2$ and $AgNO_3$) were added (to 0.2 mM) to *S. cerevisiae* (0.36 mg dry wt ml^{-1} in PIPES buffer) at 25 °C; glucose was not added. Samples were taken 5 min after metal salt additions.

Metal cations	Cell K^+ [nmol (mg dry wt) $^{-1}$]
No addition	450
Cd^{2+}	339
$Cd^{2+} + Ca^{2+}$	394
$Cd^{2+} + Zn^{2+}$	319
Co^{2+}	428
Pb^{2+}	442
Hg^{2+}	356
Cu^{2+}	122
Ag^+	111

Fig. 4. Lineweaver-Burk plots of Co^{2+} and Cd^{2+} uptake. (a) Co^{2+} uptake after glucose addition at 25 °C by *S. cerevisiae* (0.40 mg dry wt ml^{-1}) from solutions of $CoSO_4$ (○) or $CoSO_4$ plus 0.2 mM- $MgSO_4$ (●). (b) Cd^{2+} uptake after glucose addition at 25 °C by *S. cerevisiae* (0.42 mg dry wt ml^{-1}) from solutions of $CdSO_4$ (○) or $CdSO_4$ plus 0.2 mM- $MgSO_4$ (●). All data points are means of four experiments.

uptake to K^+ loss was apparent. The ability of various cations to effect K^+ release was measured (Table 1). Cu^{2+} and Ag^+ caused immediate flocculation of cells and the most extensive K^+ release. Cd^{2+} and Hg^{2+} were equally effective. Cd^{2+} -induced K^+ release was not affected by Zn^{2+} but was reduced by Ca^{2+} (Table 1; Fig. 3). Less K^+ was released in the absence of glucose (Table 1) than after glucose addition (Figs 2b and 3). When a cell suspension was pre-incubated and aerated for 6 h before Cd^{2+} addition, thus presumably exhausting endogenous energy-yielding substrates, there was little Cd^{2+} accumulation and a concurrent reduction in K^+ release (data not shown).

Kinetics of Co^{2+} and Cd^{2+} uptake

A double reciprocal plot (after Lineweaver & Burk, 1934) of the initial rates of Co^{2+} uptake at 25 °C against a range of Co^{2+} concentrations indicated two apparent affinity constants for Co^{2+} uptake (Fig. 4a). Below approximately 0.5 mM- $CoSO_4$, an apparent K_m of 77 μM and a V_{max} of 5.4 nmol Co^{2+} (mg dry wt) $^{-1}$ min $^{-1}$ were observed. Mg^{2+} was a

Table 2. *Mutual inhibition of the initial rates of metal cation uptake by S. cerevisiae from mixed solutions of CoSO₄ with other metal sulphates*

Metal sulphates were added (to 0.2 mM) after glucose addition to *S. cerevisiae* (0.43 mg dry wt ml⁻¹ in PIPES buffer) at 25 °C. Inhibition of the uptake of Co²⁺ or other metal cation is expressed as a percentage of the uptake of each cation from its respective single metal sulphate (0.2 mM) solution.

Metal cations added	Inhibition of Co ²⁺ uptake (%)	Inhibition of uptake of other metal cation (%)
Co ²⁺ + Mn ²⁺	30	61
Co ²⁺ + Cd ²⁺	25	5
Co ²⁺ + Ni ²⁺	49	43
Co ²⁺ + Zn ²⁺	73	14

Fig. 5. Effect of divalent cations on accumulation of Co²⁺ and Cd²⁺ by *S. cerevisiae* (0.43 mg dry wt ml⁻¹) at 25 °C after addition of glucose and (a) 0.2 mM-CoSO₄ (○) or (b) 0.2 mM-CdSO₄ (○), with 0.2 mM sulphates of Mg²⁺ (△), Ni²⁺ (●), Mn²⁺ (■), Zn²⁺ (□) and Ca²⁺ (▲).

competitive inhibitor of this uptake with an apparent K_i of 125 μ M. Above approximately 0.5 mM-CoSO₄, a sharp reduction of affinity and an increase in the rate of Co²⁺ uptake occurred with an apparent K_m greater than 0.1 M and a V_{max} of approximately 200 nmol Co²⁺ (mg dry wt)⁻¹ min⁻¹. The apparently 'biphasic' kinetic nature of Co²⁺ uptake resembles that shown for monovalent thallium uptake by *S. cerevisiae* (Norris *et al.*, 1976). However, the results cannot indicate whether the different affinities for Co²⁺ uptake reflect a change in the translocation process of a single uptake system or utilization of different uptake systems. Furthermore, cation concentration-dependent changes of the membrane surface potential can give an appearance of multiphasic uptake systems with Lineweaver-Burk plots of such data (Theuvsen & Borst-Pauwels, 1976).

Cd²⁺ uptake at 25 °C showed an apparent K_m of 1 mM and V_{max} of 50 nmol Cd²⁺ (mg dry wt)⁻¹ min⁻¹. Mg²⁺ was a less effective competitive inhibitor of Cd²⁺ uptake than of Co²⁺ uptake with an apparent K_i of 1.4 mM (Fig. 4b).

Specificity of Co^{2+} and Cd^{2+} uptake

The competitive inhibition of Co^{2+} uptake by Mg^{2+} and inhibition of Cd^{2+} uptake by Ca^{2+} (Fig. 3) were compared with inhibition of Co^{2+} and Cd^{2+} uptake by other cations (Fig. 5). The amount of divalent cation accumulated was similar during the simultaneous uptake of Co^{2+} and Mn^{2+} or Co^{2+} and Zn^{2+} , with weak inhibition of Co^{2+} uptake by Mn^{2+} complemented by strong inhibition of Mn^{2+} uptake by Co^{2+} , and strong inhibition of Co^{2+} uptake by Zn^{2+} complemented by weak inhibition of Zn^{2+} uptake by Co^{2+} (Table 2). Mutual inhibition of Ni^{2+} and Co^{2+} uptake from mixtures was generally equivalent for each cation. The uptake rates of Ni^{2+} , Mn^{2+} and Zn^{2+} in the absence of Co^{2+} were similar to the uptake rate of Co^{2+} in the absence of other divalent cations (data not shown). No Ca^{2+} uptake was detected from 0.2 mM or 1 mM CaSO_4 or $\text{Ca}(\text{NO}_3)_2$ in the absence or presence of Co^{2+} . Thus inhibition of Co^{2+} uptake decreased down the following series [crystal ionic radii of the cations, in nm, (after Pauling, 1960) are given in parentheses]:

$\text{Zn}^{2+}(0.074) > \text{Ni}^{2+}(0.072) > \text{Mg}^{2+}(0.065) > \text{Mn}^{2+}(0.080) > \text{Cd}^{2+}(0.097) > \text{Ca}^{2+}(0.099)$.

Inhibition of Co^{2+} uptake was most severe by cations of similar size to Co^{2+} (0.074 nm).

Inhibition of Cd^{2+} uptake by other divalent cations also appeared to be related to the ionic radii of the cations involved. The initial rate of Cd^{2+} (0.097 nm) uptake was strongly depressed by Ca^{2+} (0.099 nm) and less strongly by Mn^{2+} (0.080 nm), with the smaller metal cations having little effect at concentrations equimolar with Cd^{2+} (Fig. 5*b*). Uptake of Mn^{2+} was strongly inhibited by Cd^{2+} , which had little effect on the uptake of Zn^{2+} or Ni^{2+} .

Addition of K^+ or Na^+ (as sulphates) at concentrations equimolar with Co^{2+} or Cd^{2+} caused only slight inhibition of the rates of divalent cation uptake. Release of cell K^+ after Cd^{2+} addition was unaffected by the presence of the monovalent cations. The lack of effective inhibition of Cd^{2+} uptake by Na^+ (0.095 nm) indicated that cations would need to have a similar charge before the criterion of ionic radius could determine competition for uptake.

DISCUSSION

Preclusion of extensive loss of cation uptake activity by the use of cells soon after harvesting from growing cultures resulted in greater accumulation of metal cations than seen in earlier studies which used starved yeast from commercial sources (Fuhrmann & Rothstein, 1968; Ponta & Broda, 1970).

The competition for uptake between Co^{2+} and Ni^{2+} , Zn^{2+} or Mn^{2+} suggested that these cations shared a cation uptake system of low specificity, as proposed earlier by Fuhrmann & Rothstein (1968). Mutual inhibition of Mn^{2+} and Cd^{2+} uptake suggested that Cd^{2+} was also accumulated by this system. Cadmium exhibited some cell-metal cation interactions not shared by the other cations, such as (i) the relatively slight inhibition of Cd^{2+} uptake by equimolar concentrations of smaller divalent cations, despite the apparently higher affinity for Co^{2+} than for Cd^{2+} uptake; and (ii) the Cd^{2+} -induced K^+ release. The Cd^{2+} -induced K^+ loss resembled that observed previously with *S. cerevisiae* in the presence of Hg^{2+} ; the latter was attributed (Passow & Rothstein, 1960) to cell membrane disruption after metal cation-membrane sulphydryl group interactions. Sulphydryl groups are generally the preferred binding sites of Cd^{2+} and Hg^{2+} (Vallee & Ulmer, 1972), and Ag^+ , which promoted the most extensive K^+ release in the present experiments, forms strong complexes only with cysteine of all the amino acids (Gruen, 1975). Cd^{2+} -induced K^+ loss in the present experiments appeared to depend on some Cd^{2+} uptake. Either starving the cells of energy-yielding substrates, or addition of Ca^{2+} , depressed Cd^{2+} uptake and subsequent K^+ loss

although probably neither treatment would have affected Cd^{2+} binding to membrane-surface sulphhydryl groups. The K^+ loss thus appears similar to that seen with metal cation-induced passive permeability of mitochondrial membranes where, with 'energized' membrane conditions, some entry of Cu^{2+} into regions not accessible in a 'resting' membrane was required for extensive deformation of the membrane (Hwang, Scott & Brierley, 1972). The presence of Hg^{2+} in yeast cytoplasm after addition of HgCl_2 to cells (Murray & Kidby, 1975) has shown that Hg^{2+} uptake may occur and, as with Cd^{2+} , Hg^{2+} uptake might have occurred before the K^+ release observed after HgCl_2 addition.

The non-physiological concentrations of the metal cations used, leading to their gratuitous accumulation, should be emphasized. Cd^{2+} inhibition of Zn^{2+} uptake from $1.1 \mu\text{M}$ - ZnCl_2 by *Candida utilis* (Failla *et al.*, 1976) suggests that the essentially homologous chemistry of the elements and their compounds, rather than similarities of cation size, may be important in competition for metal cation uptake by yeasts from lower metal concentration ranges than used in the present study.

We thank Dr J. A. Taylor for discussion. This work was supported by the Science Research Council and Imperial Chemical Industries Ltd.

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