Accommodation of Yeast to Toxic Levels of Cadmium Ions

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Saccharomyces cerevisiae can accommodate to the presence of toxic levels of Cd^{2+} . This adaptation can also be induced, though to a lesser degree, by pre-growth of the yeast in $50\ \mu\text{M-Zn}^{2+}$. Growth of Cd^{2+} -adapted yeast through several passages in Cd^{2+} -free medium leads to a progressive decrease in Cd^{2+} -tolerance by the yeast, suggesting that the adaptation did not involve selection of a Cd^{2+} -resistant mutant. Chromatography on Sephadex G-75 of the soluble fraction from Cd^{2+} -adapted yeast indicated that no metallothionein-like protein was present. This suggests that the mechanism of adaptation is unlike that of the higher eukaryotes.

INTRODUCTION

The toxicity of cadmium is well documented and numerous studies of its biochemical effects have been made (for review, see Vallee & Ulmer, 1972). An adaptive response to Cd^{2+} , that protects the organism against damage by the cation, has been observed both in human tissue (Rugstad & Norseth, 1975) and in *Escherichia coli* (Mitra *et al.*, 1975). The mechanisms of adaptation differ, however. In mammals a cadmium-binding protein, metallothionein, is induced; it can bind 7 to 8 Cd^{2+} or Zn^{2+} ions and has a molecular weight of about 10000 (Kägi & Vallee, 1961). In *E. coli* no metallothionein exists and accommodation appears to involve an inhibition of Cd^{2+} uptake. Pre-treatment with Zn^{2+} also elicits the adaptive response, both in *E. coli* and in the rat (Webb, 1972).

The purpose of the present study was to ascertain whether induction of metallothionein represents a general eukaryotic mechanism of accommodation or is restricted to the higher eukaryotes. Failla & Weinberg (1977) have suggested that a metallothionein-like protein might control Zn^{2+} accumulation in yeast.

METHODS

Organism. A strain of *Saccharomyces cerevisiae* isolated in this laboratory was used. It was grown in batch culture at 30°C on a defined medium containing 40 g glucose, 10 g $(\text{NH}_4)_2\text{SO}_4$, 2 g KH_2PO_4 , 1 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 g NaCl , 0.2 g CaCl_2 , 30 mg inositol, 8 mg thiamin, 1 mg pyridoxine, 1 mg calcium pantothenate, 0.5 mg riboflavin, 50 μg biotin, 1 mg H_3BO_3 , 0.8 mg $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and 0.6 mg $(\text{NH}_4)_2\text{SO}_4 \cdot \text{FeSO}_4 \cdot 6\text{H}_2\text{O}$ in 1 l glass-distilled water. Cell concentrations were determined from turbidities measured at 590 nm.

Effect of Cd^{2+} on growth. The yeast was grown on defined medium alone, or in the presence of either $50\ \mu\text{M-Zn}^{2+}$ or $50\ \mu\text{M-Cd}^{2+}$, to a concentration of 0.5% (w/v, wet weight). Samples were then inoculated into fresh medium containing Cd^{2+} (0 to 1 mM) to a concentration of 0.01% (w/v), and incubated with shaking at 30°C . Turbidities were measured over a period of 24 h.

In a second experiment, yeast was grown in batch culture through three passages, the concentration of Cd^{2+} in the medium being doubled at each passage, from 5 to $40\ \mu\text{M}$. The yeast was then taken through

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Fig. 1. Effect of pre-growth in $50 \mu\text{M-Cd}^{2+}$ ($\circ$) or $50 \mu\text{M-Zn}^{2+}$ ($\square$) on the growth of yeast in batch culture on defined medium containing Cd^{2+} , as compared with the growth of normal yeast ($\bullet$, $\blacksquare$). Wet weights were calculated from turbidities measured at 590 nm. (a) Growth yield after 24 h, (b) yield after 9 h; yields are expressed as fractional increases in cell mass, $(M_t - M_0)/M_0$, where M_t is the mass (wet wt) at time t and M_0 the initial mass.

five passages in cadmium-free medium; at each passage a sample was removed and its growth was followed in medium containing $80 \mu\text{M-Cd}^{2+}$.

Assay of K^+ release. K^+ -loaded yeast was grown in medium enriched with 0.25 M-KCl . After being washed three times with distilled water, it was suspended to a concentration of 10% (w/v) in aqueous solutions of CdCl_2 (0 to 75 mM) or in 7.5 mM-HgCl_2 . After incubation for 1 h , the yeast suspension was centrifuged and K^+ in the supernatant was determined by flame photometry at 767 nm . K^+ was also determined in the supernatant from a suspension in 2 M-HCl that had been autoclaved for 20 min at 121°C .

Assay of Cd^{2+} exchange. ^{109}Cd -loaded yeast was prepared by culture in medium containing $5 \mu\text{M-CdCl}_2$, $0.01 \mu\text{Ci ml}^{-1}$ (carrier-free $^{109}\text{CdCl}_2$ from The Radiochemical Centre, Amersham). It was washed with distilled water, followed by 5 mM-CdCl_2 , and then suspended to a concentration of 5% (w/v) in $50 \text{ mM-N-tris(hydroxymethyl)methyl-2-aminoethane sulphonate}$, pH 6.5 , containing 5 mM-CdCl_2 and 50 mM-glucose . Samples were removed at intervals up to 60 min , filtered through Millipore filters (pore size $0.45 \mu\text{m}$) and the filtrates were counted using a Packard liquid scintillation spectrometer (Tri-Carb model 3320).

Gel filtration. Sphaeroplasts were prepared from washed ^{109}Cd -loaded yeast by the method of Cabib (1971) using crude β -glucuronidase (Sigma), and disrupted by sonication for 1 min at 50 W (Sonifier Cell Disruptor, model W185, Heat Systems-Ultrasonics, U.S.A.). Cell debris was removed by centrifugation at $5000 g$ for 10 min followed by centrifugation at $100000 g$ for 1 h . The supernatant was passed through a $70 \times 1.5 \text{ cm}$ column of Sephadex G-75 using 0.001 M-Tris/HCl , pH 8.6 , as eluting buffer (Rugstad & Norseth, 1975).

RESULTS

Effect of Zn^{2+} and Cd^{2+} pre-treatment on growth in Cd^{2+} -containing medium. Cell yields after 24 h growth in Cd^{2+} -containing medium are shown in Fig. 1(a) for both normal yeast and yeast pre-grown in the presence of $50 \mu\text{M-Cd}^{2+}$. Initial growth rates were slower for cadmium-adapted yeast at concentrations up to $500 \mu\text{M-Cd}^{2+}$, but whereas control growth began to decrease after about 8 h , that of the cadmium-adapted yeast continued in exponential phase beyond 24 h . A similar, but less pronounced effect on cell yield was observed for yeast pre-grown in $50 \mu\text{M-Zn}^{2+}$ (Fig. 1b).

The ability of cadmium-adapted yeast to grow in medium containing $80 \mu\text{M-Cd}^{2+}$ decreased after subsequent growth in cadmium-free medium (Fig. 2). The degree to which this ability was lost was related to the number of passages of the yeast through the cadmium-free medium.

Exchange of $^{109}\text{Cd}^{2+}$. Over a period of 1 h no increase in ^{109}Cd was detected in the filtrates from suspensions of ^{109}Cd -loaded yeast in 5 mM-CdCl_2 .

Effect of Cd^{2+} on membrane integrity. Hg^{2+} and Cu^{2+} damage cell membranes irreversibly (Passow & Rothstein, 1960). Because of similarities in the chemistry of Hg^{2+} and Cd^{2+} ,

Fig. 2. De-adaptation of yeast towards Cd^{2+} toxicity. Cadmium-adapted yeast was prepared by growth in batch culture through three passages, the concentration of Cd^{2+} in the medium being doubled at each passage, from 5 to 40 μM . This yeast was then grown through five passages on 100 ml cadmium-free medium. Growth of yeast removed at each passage was followed in medium containing 80 μM - Cd^{2+} (from an initial concentration of 0.025%, w/v). Growth of: unadapted yeast on 80 μM - Cd^{2+} (●); adapted yeast (○); de-adapting yeast, second (□), third (■), fourth (△) and fifth (▲) passages.

the effect of Cd^{2+} on membrane integrity was determined, $^86\text{K}^+$ release being used as a criterion of membrane damage. Small amounts of K^+ (about 5% of the total K^+) were detected in every supernatant in which Cd^{2+} was present but the amount was independent of Cd^{2+} concentration over the range 5 to 75 mM. In contrast, 7.5 mM- Hg^{2+} caused release of all the intracellular K^+ .

Gel filtration of soluble fraction from ^{109}Cd -loaded yeast. After treatment of ^{109}Cd -loaded yeast with β -glucuronidase, about 75% of the total radioactivity was found in the supernatant. After disruption of the sphaeroplasts and centrifugation, the soluble fraction contained only 9% of the total radioactivity. Gel filtration of the soluble fraction indicated that about 70% of the ^{109}Cd was bound to proteins eluted with the void volume (molecular weight > 70000). Most of the remaining ^{109}Cd eluted at V_t (total volume) suggesting that it is present as the free ion or bound to substances of low molecular weight (< 3000). No ^{109}Cd was detected at an elution volume coincident with that expected for a metallothionein of molecular weight 10000.

DISCUSSION

The results suggest that *S. cerevisiae* is similar to both mammals and bacteria in being able to accommodate to the presence of toxic levels of Cd^{2+} , and that this accommodation can also be induced by Zn^{2+} . It is unlikely that the observed resistance is caused by selection of cadmium-resistant mutants since growth in Zn^{2+} -containing medium exerts no selective pressure at the Zn^{2+} concentrations used. Moreover, cadmium-adapted yeast shows a progressive de-adaptation when grown in medium lacking Cd^{2+} . This is possibly a reflexion of the progressive dilution of intracellular Cd^{2+} that must occur during growth. The Cd^{2+} does not appear to exert its toxic effects by disruption of the yeast plasma membrane.

The absence of an inducible metallothionein suggests that the mechanism of accommodation in yeast may resemble that in *E. coli*. The observation that only 9% of the ^{109}Cd was found in the soluble fraction of yeast compares closely with the value of 10% found for *E. coli* by Mitra *et al.*, (1975). In rat liver, 85% of ^{109}Cd is bound to metallothionein (Winge & Rajagopalan, 1972).

In several other systems the size of the intracellular pool of a particular metabolite has been shown to regulate the associated transport system by transinhibition (Grabeel & Grenson, 1970; Cummins & Mitchison, 1967), and it is possible that high intracellular levels of Cd^{2+} inhibit the divalent cation transport system in yeast. The observation that

the degree of de-adaptation is related to the period of growth in cadmium-free medium supports this hypothesis. The kinetics of Cd^{2+} transport in cadmium-adapted and normal yeasts are being investigated. Since no exchange of ^{109}Cd appears to occur it is unlikely that accommodation involves efflux of the cation.

The absence of a metallothionein in *S. cerevisiae* argues against the suggestion of Failla & Weinberg (1977) that a protein of this type is involved in control of Zn^{2+} accumulation by yeasts.

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