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A comparison of the effects of chromate, molybdate and cadmium oxide on respiration in the yeast *Saccharomyces cerevisiae*

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Summary. Growth of *Saccharomyces cerevisiae* on non-fermentable medium was more sensitive to inhibition by chromate than growth on fermentable medium. Chromate was selectively toxic against oxygen uptake in cells grown in non-fermentable medium and also induced *petite* mutations. CdO demonstrated similar but lesser effects on growth and respiration. However, molybdate had little toxicity to yeast non-fermentable growth and stimulated oxygen uptake in cells grown in fermentable and non-fermentable media. These results suggest that chromate, a carcinogen, may act more directly against the mitochondria of *S. cerevisiae* than related chemical species, CdO and molybdate.

Key words: *S. cerevisiae* — Respiration — Chromate — Molybdate — CdO

Introduction

The transition elements chromium and molybdenum both possess a variety of oxidation states including the oxyanions of Cr(VI), CrO_4^{2-}, and Mo(VI), MoO_4^{2-} (Pope et al. 1980). Chromate, the hexavalent form of chromium, is a well documented carcinogen in man (Langard 1980) and can cause mutations in bacteria (Venitt and Levy 1974), yeast (Fukunaja et al. 1982) and cultured mammalian cells (Newbold et al. 1979). Molybdenum is an essential trace element in plants, animals and bacteria (Rajagopalan 1988) as it acts as a cofactor in a variety of enzymes, including bacterial nitrogenases, nitrate reductases in plants and bacteria and some hydroxylases such as milk xanthine oxidase (Bray 1988). Cadmium and chromium are toxic to growth of *Saccharomyces cerevisiae* (White and Munns 1951). Cadmium can induce genetic recombination (Fukunaja et al. 1982) and accumulate in the mitochondria of yeast cells leading to irreversible damage (Lindegren and Lindegren 1973).

It has now been established that many carcinogens cause mitochondrial mutations in *S. cerevisiae* (Egilsson et al. 1979). Some carcinogens may act primarily against mitochondria leading to genetic lesions and altered cell-surface characteristics (Wilkie and Evans 1982).

The yeast *S. cerevisiae* is particularly well suited for studying the anti-mitochondrial activities of chemicals as it is a facultative anaerobe and is therefore able to grow glycolytically, in the absence of a functional respiratory system, on a fermentable carbon source. Toxicants which selectively inhibit components of the mitochondrial system, and which would be lethal to most eukaryotic cells, are more potent in inhibiting yeast growth on a non-fermentable medium (e.g. glycerol), where mitochondrial function is essential, than on a fermentable medium (e.g. glucose) where respiration is dispensable (Wilkie 1972; Bruce et al. 1987). Chemicals which interact with mitochondrial function or DNA can also select and/or induce mitochondrial *petite* mutation. *Petite* mutants, which result from extensive deletions in the mitochondrial genome are unable to respire and can only grow glycolytically (Bernardi 1979).

These properties of *S. cerevisiae* were exploited to study and compare the differences in toxicity against mitochondrial respiration of chromate (a carcinogen), molybdate (a phosphate analogue) and cadmium oxide.

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Materials and methods

Chemicals. Molybdic acid (H_2MoO_4), potassium dichromate ($K_2Cr_2O_7$) and cadmium oxide (CdO) were obtained from BDH Chemicals Ltd, UK. H_2MoO_4 stock solutions (1 M) were dissolved in dilute ammonia. CdO (0.01 M) and $K_2Cr_2O_7$ (0.01 M) stock solutions were prepared in double distilled water. Solutions were filter-sterilized. All other reagents were of Analar grade quality.

Media. Cells were routinely grown in 1% yeast extract/2% D-glucose (YED) or 1% yeast extract/4% glycerol (YEG) media. These media were buffered with 100 mM Tris/succinate pH 6.0 when used in growth tests. *Petite* mutants were isolated on YEG medium containing 0.1% D-glucose (PDM). 2% Bactoagar was used to solidify the media (Sherman et al. 1983). All media components were obtained from Difco Laboratories.

Yeast strains. A variety of genetically marked haploid strains of *S. cerevisiae* were used in preliminary screening experiments (Table 1); a single representative strain, DB7 747 (*a, trp*$^-$, *leu*$^-$, *ura*$^-$, *his*$^-$) was used in all subsequent toxicity and oxygen electrode experiments.

Growth tests on solid medium. The effects of H_2MoO_4, $K_2Cr_2O_7$ and CdO on growth were assessed by drop inoculation of suspensions of yeast strains, using a multiple inoculation device (Wilkie 1972), onto series of YED and YEG plates containing either H_2MoO_4 concentrations of 0, 0.025, 0.05, 0.08, 0.1, 0.2 or 0.4 M; $K_2Cr_2O_7$ concentrations of 0, 0.05, 0.1, 0.25, 0.5, 1.0, 2.0 or 3.0 mM; or CdO concentrations of 0, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 or 1.0 mM. Plates were incubated at 30° C for 2 days and minimum inhibitory concentrations (mic, i.e. the concentrations of H_2MoO_4, $K_2Cr_2O_7$ and CdO required for total inhibition of growth) scored visually.

Growth tests in liquid medium. Approximately 2.5×10^7 cells of strain DBY 747 were inoculated into 250-ml conical flasks containing 25 ml YED and YEG liquid media and various concentrations of H_2MoO_4, $K_2Cr_2O_7$, and CdO. The flasks were incubated at 30° C in an orbital shaker and total cell counts made every 6 h using a haemocytometer.

Isolation and identification of petite *mutants. Petite* mutants were initially identified by their small size (approximately 1 mm diameter) on PDM agar after 4–5 days incubation at 30° C, and secondly by their inability to grow when transferred to YEG medium.

Respiration rates. Rates of oxygen uptake by cells at 30° C were measured in a Clark-type oxygen electrode coupled to a pen recorder (Estabrook 1967). 2.5×10^7 cells of strain DBY 747 were inoculated into 25 ml YED or YEG liquid media containing either (a) 0, 1.0, 5.0 or 10.0 mM H_2MoO_4, (b) 0, 0.1, 0.5 or 1.0 mM $K_2Cr_2O_7$, or (c) 0, 0.005, 0.01 or 0.02 mM CdO, and incubated at 30° C in an orbital shaker for approximately 18 h. Cells were counted and resuspended at 10^7 cells · ml^{-1} in the electrode chamber and respiration rates recorded polarographically. Cell suspensions were serially diluted and plated, in triplicate, onto YED agar to determine cell viability.

Table 1. Differences in sensitivity to H_2MoO_4, $K_2Cr_2O_7$ and CdO of 11 haploid strains of *S. cerevisiae* on fermentable (YED) and non-fermentable (YEG) media

Strain	Minimum inhibitory concentrations (mM)					
	H_2MoO_4		$K_2Cr_2O_7$		CdO	
	YED	YEG	YED	YEG	YED	YEG
D26 (*a, ade*$^-$)	100	100	1.0	0.1	0.8	0.4
657 (*a, lys*$^-$)	100	100	1.0	0.1	0.6	0.05
6-81 (*a, met*$^-$, *arg*$^-$)	100	100	1.0	0.1	0.4	0.1
DBY 747	100	100	1.0	0.1	0.2	0.1
D22 (*a, ade*$^-$)	100	100	1.0	0.1	0.6	0.1
D75 (*a, met*$^-$, *arg*$^-$)	100	100	1.0	0.1	1.0	0.6
D6 (*a, arg*$^-$, *ilu*$^-$)	25	25	1.0	0.1	1.0	0.8
22-701 (*a, ade*$^-$)	80	100	0.5	0.1	1.0	0.8
B27 (*α, met*$^-$, *arg*$^-$)	200	100	1.0	0.1	0.2	0.1
51782b (*a*)	100	100	1.0	0.1	0.6	0.1
2180A (*a*)	100	100	1.0	0.1	1.0	0.8
Mean values	100	90	1.04	0.10	0.67	0.35

Minimum inhibitory concentrations were assessed as described in Materials and methods

Results

Growth tests

Molybdate was relatively non-toxic to *S. cerevisiae* compared to chromate and CdO, with growth occurring at concentrations up to 100 mM H_2MoO_4 (Table 1). No significant differences in minimum inhibitory concentration (mic) were observed between the growth of yeast strains on fermentable (YED) and non-fermentable (YEG) solid media (Table 1), and in strain DBY 747 in liquid media (Fig. 1a). These results imply that molybdate has no anti-mitochondrial effects.

In contrast chromate showed clear anti-mitochondrial activities, as the sensitivities of yeast strains grown on YEG solid medium were approximately 10-times greater (mean mic = 0.10 mM $K_2Cr_2O_7$) than those grown on YED solid medium (mean mic = 1.0 mM) (Table 1). This result was confirmed in growth tests using strain DBY 747 in liquid YED and YEG media (Fig. 1b).

CdO had similar but lesser effects than chromate on the growth of *S. cerevisiae*. The differences in sensitivity to CdO to yeast growth on YEG and YED solid media were approximately two-fold, with the average mic on YEG medium being 0.35 mM and on YED 0.67 mM (Table 1). The smaller anti-mitochondrial effect produced by CdO, compared to chromate, was further illustrated by the similarity in sensitivity of strain DBY 747 grown in YEG and YED liquid media (Fig. 1c).

The discrepancies between the inhibitory con-

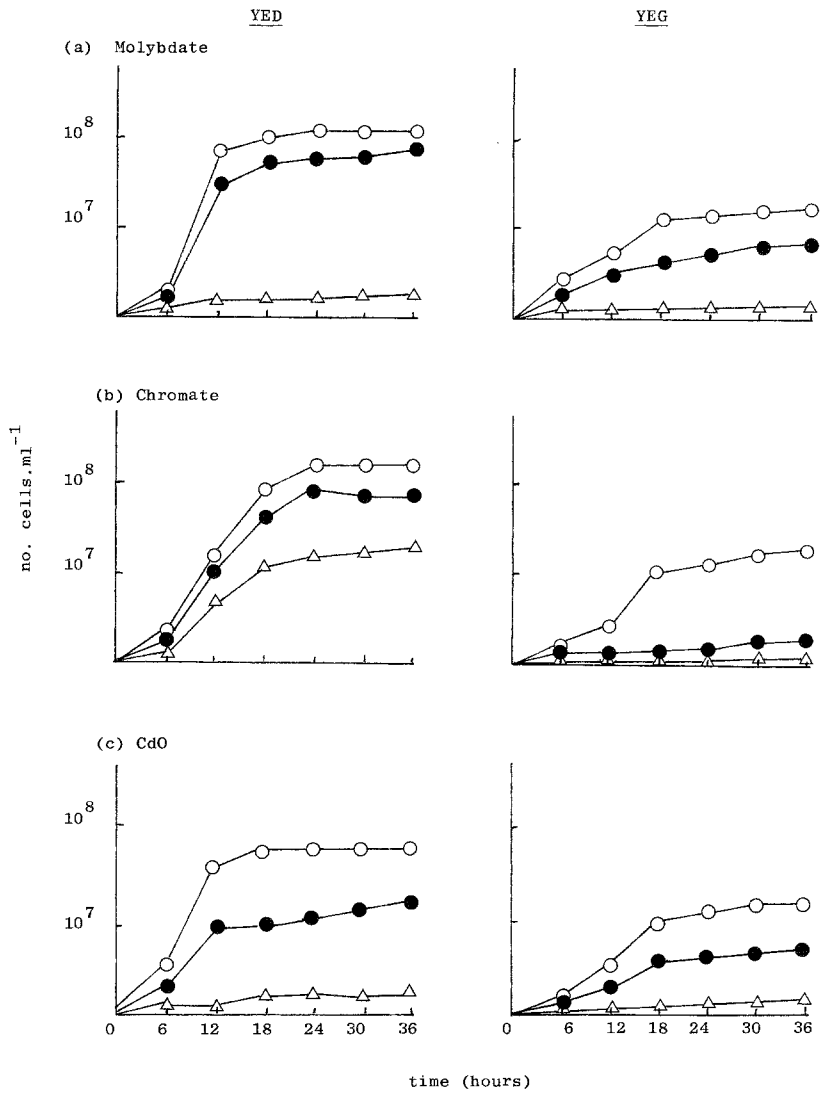

Fig. 1. Effects of **(a)** molybdate, **(b)** chromate and **(c)** CdO on growth of *S. cerevisiae* strain DBY 747. Cells were grown for 36 h in YED or YEG liquid media containing **(a)** 0 (○), 1.0 (●) and 5.0 mM (△) H_2MoO_4; **(b)** 0 (○), 0.5 (●) and 1.0 mM (△) $K_2Cr_2O_7$; or **(c)** 0 (○), 0.005 (●) and 0.02 mM (△) CdO. Results plotted are representative of two experiments

centrations of molybdate and CdO on solid and in liquid media (Table 1 and Fig. 1), with yeast growth being approximately 100-times more sensitive to molybdate and CdO in liquid media, may be caused by binding of the metals to constituents of the solid medium (Gadd 1983).

Cell viability

The anti-mitochondrial effects of molybdate, chromate and CdO observed in preliminary growth tests (Table 1) were further confirmed by the differences in cell viability of strain DBY 747 to each species.

Molybdate concentrations (5.0 mM) inhibitory to growth (Fig. 1a) cause little change in cell viability. 10.0 mM H_2MoO_4 still only produced an approximately 50% reduction in viability in both YED and YEG media. Cells grown in YEG medium were slightly molybdate-sensitive than those grown in YED medium possibly indicating some anti-mitochondrial activity (Fig. 2a).

The viability of cells exposed to 1.0 mM $K_2Cr_2O_7$ was reduced by 60% in YEG cultures but only by 20% in YED cultures (Fig. 2b). However, almost 80% of cells exposed to the growth inhibitory concentration of 0.5 mM $K_2Cr_2O_7$ (Fig. 1b) were still viable (Fig. 2b). The concentrations of CdO required to prevent growth and cause cell death were similar (Figs. 1c and 2c).

Petite *mutant frequency*

The frequency of *petite* mutants slightly increased

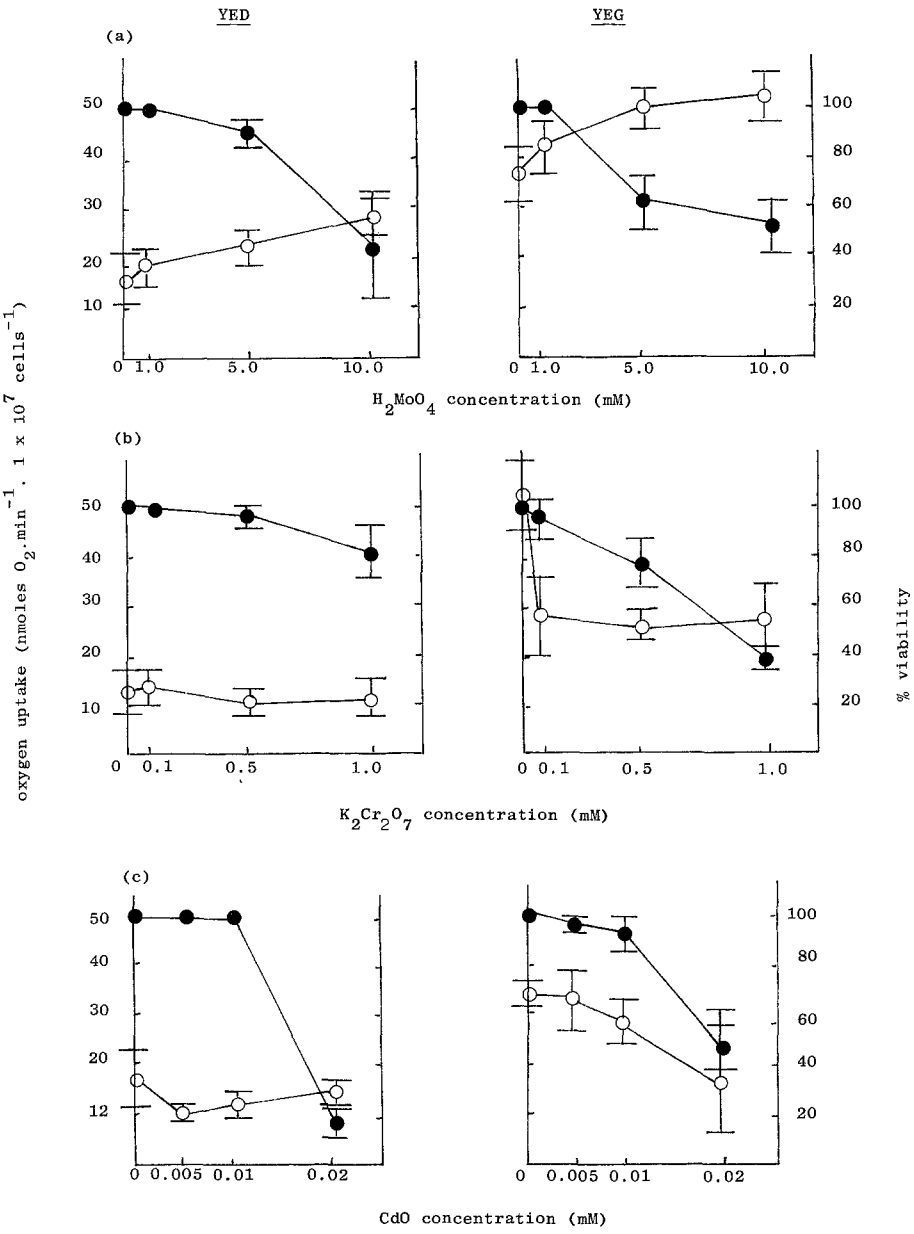

Fig. 2. Effects of increasing concentrations of **(a)** H_2MoO_4, **(b)** $K_2Cr_2O_7$, and **(c)** CdO on oxygen uptake (○) and cell viability (●) of *S. cerevisiae* strain DBY 747 in fermentable (YED) and non-fermentable (YEG) media. Results plotted represent rate of oxyen uptake (nmol·min^{-1}) by 10^7 cells, and percentage viability, and are the means of 3–8 experiments ± standard deviations

in cultures of strain DBY 747 containing molybdate, chromate and CdO (Table 2) when compared to control cultures. Both molybdate and chromate appeared to induce *petite* mutants, as their frequency increased in cells grown in YEG medium containing up to 10 mM H_2MoO_4 or 1.0 mM $K_2Cr_2O_7$ (Table 2a and b). A different response was observed in CdO-treated cultures, where an approximately six-fold increase in *petite* mutant frequency occurred in YED medium containing 0.02 mM CdO. No change was observed in the *petite* mutant frequency of YEG cultures (Table 2c).

Respiration rates

The respiration rate of whole cells grown for 18 h in YED medium containing 10.0 mM H_2MoO_4 was stimulated 1.6-fold and those grown in YEG medium containing 10.0 mM H_2MoO_4 1.4-fold. These increases in oxygen uptake correlated with decreases in cell viability (Fig. 2a). A similar stimulatory effect was previously observed with another phosphate analogue vanadate (Henderson et al. 1989).

The respiratory activity of cells grown for 18 h in YED medium containing either 1.0 mM

Table 2. Effect of increasing concentrations of **(a)** H_2MoO_4, **(b)** $K_2Cr_2O_7$ and **(c)** CdO on *petite* mutant frequency

Compound	Medium	Conc (mM)	No. colonies	No. *petite*	*Petite* (%)
(a) H_2MoO_4	YED	0	4162	54	1.3
		1.0	2968	64	2.2 (1.7)
		5.0	1734	30	1.7 (1.3)
		10.0	756	14	1.8 (1.4)
	YEG	0	6822	35	0.5
		1.0	2702	14	0.5
		5.0	1063	14	1.3 (2.6)
		10.0	979	17	1.7 (3.4)
(b) $K_2Cr_2O_7$	YED	0	2534	32	1.2
		0.1	3163	83	2.6 (2.2)
		0.5	3625	91	2.5 (2.1)
		1.0	1909	45	2.4 (2.0)
	YEG	0	4501	27	0.6
		0.1	2617	34	1.3 (2.2)
		0.5	2895	49	1.7 (2.8)
		1.0	1001	16	1.6 (2.6)
(c) CdO	YED	0	4174	67	1.6
		0.005	2097	60	2.8 (1.8)
		0.01	1476	34	2.3 (1.4)
		0.02	312	33	10.6 (6.6)
	YEG	0	4018	41	1.0
		0.005	3685	26	0.7
		0.01	2386	26	1.1
		0.02	1854	22	1.2 (1.2)

Samples were removed from stationary phase cultures of strain DBY 747 grown in YED and YEG liquid media containing H_2MoO_4, $K_2Cr_2O_7$ and CdO, the cell density measured using a haemocytometer and 0.1 ml of a serial dilution of each of the suspensions plated in triplicate onto PDM agar for determination of *petite* frequencies. The numbers in parentheses represent × spontaneous rate

$K_2Cr_2O_7$ or 20.0 μM CdO was similar to that of the control cultures. However, oxygen uptake of cells grown in YEG medium containing either 0.1 mM $K_2Cr_2O_7$ or 0.02 mM CdO decreased by approximately 50% of the control rate (Fig. 2b and c).

The decrease in oxygen uptake in cells grown in YEG medium containing CdO correlated with a decrease in cell viability (Fig. 2c). However, the respiration rate was reduced in cells grown in YEG medium containing 0.1 mM $K_2Cr_2O_7$ (Fig. 2b), a concentration of chromate at which most cells are viable and the mic. in YEG medium.

Discussion

Chromate caused the most pronounced anti-mitochondrial effects in growth tests (Table 1 and Fig. 1b) and in oxygen-uptake experiments (Fig. 2b). Its analogue, molybdate, showed no significant effects on mitochondrial activity in growth tests (Table 1 and Fig. 1a) but stimulated oxygen uptake in whole cells (Fig. 2a), and like chromate appeared to induce *petite* mutations (Table 2). Molybdate may act at a variety of intracellular sites (Passow et al. 1961) like its structural analogues vanadate and phosphate, which also stimulate respiration in *S. cerevisiae* (Henderson et al. 1989).

Molybdate and chromate probably enter cells by the anion transport mechanisms responsible for the uptake of phosphate and dicarboxylate (Hammond and Foulkes 1986). Inside cells the strongly oxidising hexavalent chromate ion is reduced to Cr(III) by NAD(P)H (Gruber and Jennette 1978) and glutathione (Debetto et al. 1988).

As Cr(III) is thought to be mutagenic and the mediator of carcinogenesis, the reduction of Cr(IV) may be the first step in cellular toxicity (Tsapakos et al. 1981). Cr(III) induces carcinogenesis by reacting with nuclear DNA causing DNA-protein cross-links (Wedrychowski et al. (1985), DNA-DNA cross-links (Tsapakos et al. 1981) and DNA single-strand breaks (Kawanishi et al. 1986).

The clear differences in sensitivity of *S. cerevisiae* to chromate on fermentable and non-fermentable media (Table 1 and Fig. 1b) and the inhibition of actively respiring cells in YEG me-

dium clearly demonstrates the selective toxicity of chromate against mitochondrial function. Chromate, or the reduced form Cr(III), may act at several sites in the mitochondrion to inhibit respiration and induce *petite* mutants as it may prevent mitochondrial protein synthesis (Egilsson et al. 1979) and accumulate in the mitochondrial matrix (Hammond and Foulkes 1986).

Cadmium is extremely toxic to yeast cells (White and Munns 1951), but had less selective anti-mitochondrial activity than the relatively less toxic chromate. This was unexpected as, although CdO is not known to be carcinogenic, it interferes with mitochondrial function by inducing damage to the mitochondrial matrix (Lindegren and Lindegren 1973), inhibiting cytochrome *c* oxidase activity (Muller and Stacey 1988) and by disrupting the inner mitochondrial membrane making it impermeable to cations and protons (Skul'sky et al. 1988).

In conclusion, the evidence presented here indicates that the carcinogen chromate has greater selective toxicity against mitochondria of *S. cerevisiae* than molybdate or cadmium oxide. Mitochondria may therefore have an important role in cells as primary target sites for carcinogens.

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