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Effect of Cysteine on Chromate Resistance in the Yeast *Saccharomyces cerevisiae*

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Saccharomyces cerevisiae strains defective in cysteine biosynthesis were more sensitive to chromate than their corresponding isogenic prototrophic strains. Chromate sensitivity of the cysteine-dependent strains was reduced when L-cysteine was added to the growth medium. The effect of L-cysteine on chromate sensitivity was, however, not specific to the cysteine-dependent strains but was general to all strains tested. That is, addition of L-cysteine to the growth medium containing chromate caused an increase of colony-forming ability of chromate-sensitive, normal and chromate-resistant strains. It was also shown that L-cysteine nonbiologically consumed chromate. From these results, we conclude that cysteine plays an essential role in defence of cells against chromate. We contend that cysteine chemically reduces toxic hexavalent chromium to less toxic trivalent chromium; detoxication of chromate by this mechanism takes place intracellularly as well as extracellularly.

Keywords——*S. cerevisiae*; resistance; detoxication; chromate; cysteine

Introduction

Despite the fact that chromium is a major environmental pollutant its effects at the cellular and subcellular levels are not well understood. It is known that hexavalent chromium, present as chromate ion in water, is highly toxic to living organisms due to its strong oxidizing activity. To study the cellular effects of chromate and biological mechanisms of chromate resistance, we have been using a eucaryotic microbe, the yeast *S. cerevisiae*, as an experimental organism. Previously, we have described mutants resistant or sensitive to chromate.[1] One of the loci giving rise to chromate-sensitive mutations was identified as *LYS7*, which codes for homocitrate dehydrase, which is thought to be identical to homoaconitate hydratase (EC 4.2.1.36).[2] Therefore, it is suspected that this enzyme is involved in detoxication of chromate in the yeast cell.

In the course of the above-mentioned study,[1] we have examined some strains in our stock collection for response to chromate. Although most strains showed indistinguishable levels of sensitivity to chromate, the level assigned as "wild type" or "normal," a few strains were more sensitive or resistant than the normal strains. However, responses to chromate were not correlated to defined mutations or phenotypes, except for cysteine dependence. That is, strains dependent on cysteine were all sensitive to chromate. In this report we describe this observation and additional evidence which together lead to the conclusion that cysteine detoxifies chromate.

Materials and Methods

Yeast Strains——Strains used in this study are listed in Table I. Strains containing *cys1 cys3* or *cys2* required

TABLE I. *S. cerevisiae* Strains

Strains	Genotype	Reference
D273-10	α wild type	3
BO6-18B	α *his5-2 leu2-1 met8-1*	1
JW2-13A	α *cys1-3 cys3-1 CUP1*	4
JW4-5C	**a** *cys1-3 cys3-1 CUP1*	4
JW1-1C	α *cys2-1 CUP1*	4
JW1-2C	**a** *cys2-1 CUP1*	4
OK184-1C	α *cys1-3 cys3-1*	5
OK200-1C	**a** *cys2-1*	This study
WT	**a** wild type[a)]	6
No. 13	**a** *met17*[a)]	6
No. 16	**a** *met2 met17*[a)]	6
No. 17	**a** *met17*[a)]	6
26S-3	α *chs1 his5-2 leu2-1 met8-1*	1*a*
26S-7	α *chs2 his5-2 leu2-1 met8-1*	1*a*
26S-13	α *chs3 his5-2 leu2-1 met8-1*	1*a*
26S-18	α *chs5 his5-2 leu2-1 met8-1*	1*a*
26S-19	α *chs6 his5-2 leu2-1 met8-1*	1*a*
26R-1	α *CHR1-1 his5-2 leu2-1 met8-1*	1*b*

a) These strains were suspected to contain a chromate-resistant mutation (see the text).

cysteine for growth; the *cys1* and *cys2* mutations cause defects in serine acetyltransferase (EC 2.3.1.30),[7] and the *cys3* mutation causes deficiency of γ-cystathionase (EC 4.4.1.1).[5] Strains OK184-1C and OK200-1C were isogenic with strain D273-10B except for cysteine dependence (see Results).

Strains No. 13, 16 and 17 were derived from a prototrophic strain, WT, by ultraviolet (UV) mutagenesis. They were isolated as methionine auxotrophs and then found to be able to grow if either methionine or cysteine was supplied.[6] These mutants were deficient in *O*-acetylserine and *O*-acetylhomoserine sulfhydrylase (EC 4.2.99.10); strain NO. 16 was suggested to contain an additional defect in synthesis of *O*-acetylhomoserine.[8] The mutations responsible were shown to be *met17* and *met2*, respectively.[9] The *met2* mutation confers deficiency of homoserine acetyltransferase (EC 2.3.1.31).[10]

Strains sensitive or resistant to chromate derived from strain BO6-18B were described previously.[1]

Growth Media——YPD medium contained 1% yeast extract, 2% peptone and 2% D-glucose.[11] The medium was solidified by 2% agar. To make chromate-containing medium, an appropriate amount of CrO_3 (10 mM in water) was added to YPD medium after autoclaving and cooling to 40—50 °C. For testing growth requirements the synthetic medium described by Wickerham[12] was used as a minimal medium.

Assay of Chromate Resistance——Two methods for evaluation of resistance were used.[1] One was comparison of growth inhibition zone. Cells of strains were streaked radially on an agar YPD plate, a small filter paper was placed at the center of the plate, an aliquot (20 μl) of CrO_3 solution (1 M) was applied to the filter paper, and then the plate was incubated at 30 °C for 5 d. The other was comparison of colony-forming ability. Cells were spread on agar YPD plates containing different concentrations of CrO_3, and the plates were incubated at 30 °C for 5 d. The number of colonies on each plate relative to that on the plate without CrO_3 was taken as an indication of resistance.

Assay of Chromate——Chromate (Cr(VI)) was assayed by a colorimetric method using diphenylcarbazide (DPC).[13]

Chemicals——Yeast extract and peptone were products of Difco Laboratories, Detroit, Michigan, U.S.A. CrO_3 and amino acids were purchased from Wako Pure Chemicals Industries, Ltd., Osaka, Japan and Nakarai Chemicals, Ltd., Kyoto, Japan, respectively. DPC was a product of Ishizu Pharmaceutical Co., Ltd., Osaka, Japan.

Results

Some of our stock strains were examined for response to chromate by the filter paper disc method. Although a majority of them showed about the same level of sensitivity, the level assigned as "wild type" or "normal," a few were distinctly more resistant or sensitive than the normal strains. These different responses did not correlate to defined phenotypes or mutations, or both, except for those described below.

TABLE II. Chromate Sensitivity of the Cysteine-Dependent Strains

Strain	Remark	Sensitivity index[a)]
BO6-18B	Control	1.0
D273-10B	Wild type	0.9—1.1
JW4-5C	*cys1-3 cys3-1*	1.3—1.4
JW2-13A	*cys1-3 cys3-1*	1.3—1.7
JW1-2C	*cys2-1*	1.3—1.7
JW1-1C	*cys2-1*	1.4—1.7
OK184-1C	*cys1-3 cys3-1*	1.3—1.4
OK200-1C	*cys2-1*	1.3—1.4

a) Chromate sensitivity was examined by the filter paper disc method. The sensitivity index was calculated by dividing the distance to the growth edge of test strain by that of the control (BO6-18B).

Strains JW2-13A, JW4-5C, JW1-1C and JW1-2C, all of which required cysteine for growth, were sensitive to chromate (Table II). Since these strains were not isogenic and, moreover, contained the *CUP1* mutation (resistance to copper), we introduced the mutations responsible for cysteine dependence into a wild-type genetic background. To do so, we crossed strains JW4-5C and JW1-2C with a prototrophic strain, D273-10B. A representative cysteine-dependent progeny from each cross was again crossed to strain D273-10B. This procedure was repeated for five generations to obtain OK184-1C and OK200-1C; thus, these strains were considered to be isogenic with D273-10B, except for cysteine dependence. Strains OK184-1C and OK200-1C were free of the *CUP1* mutation. As shown in Table II, these cysteine-dependent strains were more sensitive to chromate than the isogenic prototroph, D273-10B, and a control strain, BO6-18B.

Chromate sensitivity of the cysteine-dependent strains was also demonstrated by the colony-forming ability method (Fig. 1a). Another set of isogenic strains differing in cysteine dependence showed the same tendency (Fig. 1b); *i.e.*, cysteine- or methionine-requiring mutants (No. 13, 15 and 17) were more sensitive to chromate than their parent strain WT. It was noted that strain WT was more resistant to chromate than strain BO6-18B, indicating that it contained a chromate-resistant mutation. If this were the case, we could conclude that chromate sensitivity due to cysteine dependence is epistatic over chromate resistance due to the mutation.

It became evident that cysteine-dependent strains were sensitive to chromate regardless of the loci where mutations arose. From these results, we concluded that cysteine plays an essential role in maintaining the normal level of chromate resistance in yeast. To support this conclusion, we carried out the following experiment. Strains OK184-1C and OK200-1C were spread on chromate-containing medium in the presence of various concentrations of L-cysteine, and incubated at 30 °C for 5 d, then colony-forming ability was estimated (Fig. 2). Strain D273-10B was included for comparison. Colony-forming ability of the cysteine-dependent strains increased with increase of added L-cysteine, as we expected. However, it was unexpectedly found that the colony-forming ability of strain D273-10B, which did not require cysteine, was also increased by addition of L-cysteine.

In order to examine further the effect of L-cysteine in reducing chromate toxicity, we carried out a similar experiment using a set of isogenic strains having different responses to chromate. The results are summarized in Fig. 3. It was apparent that L-cysteine caused increase of colony-forming ability, as we expected. However, such an effect was observed only when L-cysteine exceeded a certain level. Surprisingly, below this level, L-cysteine caused a slight but consistent decrease of colony-forming ability; this effect of L-cysteine was more obvious when the chromate concentration was lower and when the chromate sensitivity of the

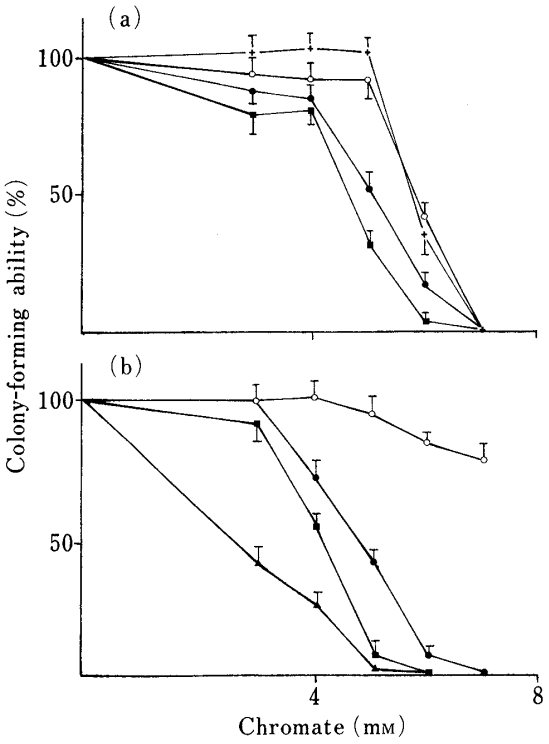

Fig. 1. Effect of Cysteine Dependence on Chromate Sensitivity

Appropriate numbers (about 200 per plate) of cells were spread on YPD medium containing various concentrations of chromate, and incubated at 30 °C for 5 d, then colonies were counted. Each point represents the average of three plates; vertical lines represent standard deviations. Colony-forming ability on medium lacking chromate was taken as 100%.

(a) Cysteine-dependent strains OK184-1C (●) and OK200-1C (■) were compared with the putative isogenic prototroph, D273-10B (○). Strain BO6-18B (+) was included as a control. (b) Cysteine- or methionine-dependent mutants, No. 13 (▲), No. 16 (■) and No. 17 (●) were compared with the parent strain, WT (○).

TABLE III. Consumption of Chromate by Incubation with L-Cysteine

Treatment[a]	A_{540}[b] after incubation of			
	0 h	23 h	46 h	70 h
None	0.44 ± 0.07	0.54 ± 0.03	0.54 ± 0.04	0.52 ± 0.03
	(100)	(123)	(123)	(118)
Cysteine	NT[c]	0	0	0
		(0)	(0)	(0)
Leucine	0.48 ± 0.05	0.55 ± 0.02	0.54 ± 0.01	0.53 ± 0.01
	(100)	(115)	(113)	(110)
Methionine	0.45 ± 0.09	0.52 ± 0.02	0.51 ± 0.01	0.47 ± 0.01
	(100)	(115)	(113)	(104)
Glucose	0.47 ± 0.07	0.50 ± 0.01	0.48 ± 0.02	0.42 ± 0.01
	(100)	(106)	(102)	(89)
YPD	0.54 ± 0.03	0.42 ± 0.02	0.32 ± 0.02	0.26 ± 0.01
	(100)	(78)	(59)	(48)
YPD + cysteine	NT[c]	0	0	0
		(0)	(0)	(0)
YPD + leucine	0.55 ± 0.02	0.43 ± 0.02	0.33 ± 0.01	0.26 ± 0.01
	(100)	(78)	(60)	(47)
YPD + methionine	0.54 ± 0.01	0.45 ± 0.02	0.33 ± 0.01	0.26 ± 0.02
	(100)	(83)	(61)	(48)

a) Aliquots of 20 μl of 10 mM CrO_3 were added to 1 ml portions of the following: cysteine = 10 mM L-cysteine, leucine = 14 mM L-leucine, methionine = 8 mM L-methionine and glucose = 2% D-glucose. YPD consisted of 0.5% yeast extract, 1% peptone and 1% D-glucose (half the concentration of YPD medium). *b*) The mixtures containing chromate were incubated at 30 °C, and 0.1 ml aliquots were withdrawn at the indicated intervals. The samples were added to 1.0 ml water, and then placed in a water-bath at 15 °C followed by addition of 0.5 ml of 1% (w/v) DPC in 50% acetone and 0.5 ml of 50% sulfuric acid. After incubation of the mixtures at 15 °C for 5 min, the absorption at 540 nm was measured. The average and standard deviation obtained from four independent experiments are presented. Figures in parentheses indicate % A_{540} (chromate) remaining. *c*) Not tested; absorption reached 0 within 10 min after mixing chromate and L-cysteine.

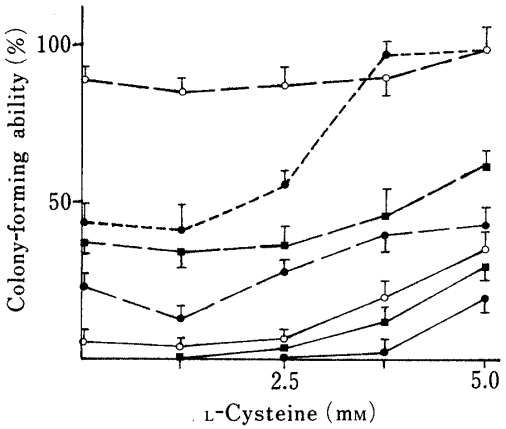

Fig. 2. Effect of Exogenous L-Cysteine on Chromate Sensitivity of the Cysteine-Dependent Strains

Cells were spread on chromate-containing medium in the presence of various concentrations of L-cysteine; chromate concentrations were 6 mM (——), 5 mM (— —) and 4 mM (– – – –). Colony-forming ability was estimated as described in Fig. 1. Strains OK184-1C (●) and OK200-1C (■) were compared with strain D273-10B (○).

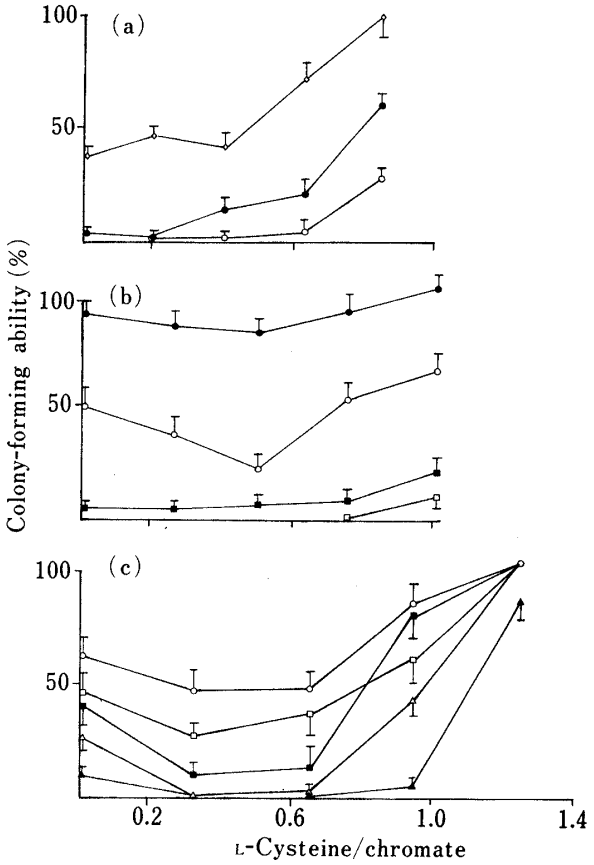

Fig. 3. Effect of Exogenous L-Cysteine on Chromate Sensitivity of Various Strains

Experimental procedures were as described in Fig. 2; chromate concentrations were 6 mM (a), 5 mM (b) and 4 mM (c). Chromate-sensitive strains 26S-3 (□), 26S-7 (○), 26S-13 (▲), 26S-18 (■), 26S-19 (△) and a chromate-resistant strain 26R-1 (◇) were examined together with their parent strain BO6-18B (●).

strain was higher. In general, the least colony-forming ability was seen at a mole ratio (L-cysteine/chromate) of 0.2 to 0.6; the higher the chromate concentration, the lower the value. It should be noted that cysteine present in YPD medium was not taken into account in calculating the above value.

In order to confirm the above-mentioned effect of L-cysteine, chromate was treated with L-cysteine as well as some other organic compounds (Table III). It was clear that L-cysteine caused instantaneous consumption of chromate, whereas L-leucine, L-methionine and D-glucose did not consume chromate at all. Loss of chromate in the presence of YPD medium could, in part, be attributed to cysteine present in the medium.

Discussion

Plasmid-mediated chromate resistance has been reported in the bacterium *Streptococus lactis*,[14] but the molecular mechanism of resistance is not understood. In *Escherichia coli*, a chromosomal mutation, *cysA*, confers chromate resistance.[15] Breton and Surdin-Kerjan[16] have obtained chromate-resistant mutants in the yeast *S. cerevisiae* and have attributed the resistance to impairment of the sulfate permeation system, which also mediates uptake of

chromate. On the other hand, Ono and Weng[1b] have claimed that chromate resistant mutants are only partially defective in chromate uptake. Here, we have demonstrated that cysteine is involved in resistance to chromate. We have clearly shown that interaction of cysteine with chromate is nonbiological in nature (Table III); however, the possibility that cells have enzyme(s) facilitating the reaction has not been excluded.

It is rather surprising that cysteine at low concentrations somewhat enhances the toxicity of chromate (Fig. 3). There are two possible explanations for this effect of cysteine; (1) cysteine acts on cells to stimulate uptake of chromate and (2) cysteine forms a complex with chromate to make it more able to permeate into the cell. Our contention is that formation of the putative complex takes place in the presence of cysteine and chromate at a certain mole ratio. However, further studies are needed to confirm this.

In studies of heavy metal resistance of *S. cerevisiae*, selection of induced mutations has been a common practice. Mutants resistant to copper,[17] methylmercury[18] and chromate[1b,16] have been reported. Mutants sensitive to chromate have also been obtained by screening of mutagenized cells.[1a] It has been found that methylmercury-resistant mutants are deficient in methionine due to mutations in the *MET2* and *MET15* loci[18] and that some chromate-sensitive mutants are deficient in lysine due to mutations in the *LYS7* locus.[1a] These observations together with those described in this report indicate that resistance to heavy metals is intimately related to cellular metabolism. It is natural to assume that metabolism of heavy metals is, at least partly, mediated by normal cellular metabolism. Therefore, it is expected that further examination of the relation between defined mutations and responses to heavy metals will provide useful information on biological defence mechanisms as well as on the metabolism of heavy metals in the cell.

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