[Chem. Pharm. Bull. 33(4)1571—1575(1985)]

# Effects of Methanol and Magnesium Chloride on the Induction of Respiration-Deficient Mutants in Yeast by Metal Ions

HIROSHI HAMADA,[a] NORIKO TOSHIMITSU,[a] MISAO KOJIMA,*,[a] and TAMOTSU MORITA[b]

*Faculty of Pharmaceutical Sciences, Fukuoka University,[a] Jonan-ku Fukuoka 814-01, Japan and Shizuoka College of Pharmacy,[b] Oshika, Shizuoka 422, Japan*

(Received July 11, 1984)

The effects of methanol and magnesium chloride on the induction of cytoplasmic respiration-deficient (RD or petite) mutants of yeast, *Saccharomyces cerevisiae*, by metal compounds were investigated. $ZnCl_2$, $HgCl_2$, $NiCl_2$, $CdCl_2$ and $MnCl_2$ induced 1.8—2.2% RD mutants at concentrations where almost no growth inhibition was observed. Induction of RD mutants by these metal compounds decreased to the spontaneous level (1.4%) in 4% methanol. The effect of methanol was weaker on RD mutant induction by $PbCl_2$ and $CuCl_2$. The induction of RD mutants by $CoCl_2$ was not affected by methanol. $NiCl_2$ and $CdCl_2$ were strongly mutagenic at higher concentrations, and the mutagenicity of these metal compounds was also repressed by 4% methanol. The frequency of RD mutants induced in the presence of $1 \times 10^{-2}$ M $MnCl_2$ was increased from 13.5 to 60.7% by the addition of methanol. This increase of the induction of RD mutants in the presence of $MnCl_2$ and methanol was antagonized almost completely by the addition of $MgCl_2$ to the culture medium. The same effect of $MgCl_2$ was observed in the absence of methanol. The effects of methanol and $MgCl_2$ on the induction of RD mutants in yeast are discussed.

**Keywords**——yeast; respiration-deficient mutation; metal ion; manganese ion; methanol-effect; magnesium-effect; mitochondrial DNA

Alcohols such as methanol and ethanol have been widely used as solvents for samples in mutagenicity tests. Though the physiological effects of alcohols have been studied in methanol-utilizing yeasts[1] and other yeasts for alcohol fermentation,[2] the mutagenic effects of alcohols on yeast cells are still obscure. We have already reported that methanol itself did not induce cytoplasmic respiration-deficient (RD or petite) mutants but prevented the induction of cytoplasmic RD mutants by aromatic alcohols[3] and acriflavine[4] in yeast, *Saccharomyces cerevisiae*. Methanol has been shown to decrease the uptake of acriflavine by yeast cells.[4]

The induction of RD mutants by metal ions such as $Ni^{2+}$, $Cd^{2+}$, $Mn^{2+}$, $Cu^{2+}$ and $Co^{2+}$ at relatively low and virtually nontoxic concentrations was observed in *S. cerevisiae*.[5-8] In the present paper, the effects of methanol on the induction of RD mutants by metal ions were examined using *S. cerevisiae*. Methanol prevented the induction of RD mutants by most of the metal ions tested, while it enhanced the induction of the mutants by $Mn^{2+}$. The increase of the induction of RD mutants in the presence of $Mn^{2+}$ and methanol was counteracted almost completely by the addition of $Mg^{2+}$. These results provide some information regarding the mechanism of the effects of methanol on the induction of RD mutants in yeast.

## Materials and Methods

**Strain**——*Saccharomyces cerevisiae* ATCC 26422 (*S. sake* Kyokai No. 7) was used.

NII-Electronic Library Service

**Chemicals**——Methanol was purchased from Nakarai Chemicals Co., Ltd. Metal compounds were commercial preparations of guaranteed reagent grade.

**Cultivation and Detection of RD Mutants**——Yeast cells precultured on Ogur's medium,[9] which contained 0.35% polypeptone, 0.3% yeast extract, 2% glucose, 2% $KH_2PO_4$, 0.1% $MgSO_4 \cdot 7H_2O$ and 0.1% $(NH_4)_2SO_4$, were inoculated into 10 ml of fresh Ogur's medium at $1 \times 10^5$ cells/ml. Each metal compound and 4% methanol, which did not show any mutagenic or growth-inhibitory effects on the yeast cells,[3] were added at the start of the incubation. After a 24-h incubation with shaking at 30 °C, cells were washed and diluted with sterilized water and spread onto Ogur's agar plates to give about 200 colonies per plate. After incubation at 30 °C for 2 d, colonies were scored for survivals and RD mutation by the tetrazolium salt overlay method.[10] Both completely white and sectored colonies were counted as RD mutants. Results were expressed as averages of at least three independent experiments with 5 plates.

## Results

### Effects of Metal Ions on Yeast Growth

When yeast cells were grown in Ogur's liquid medium at 30 °C for 24 h without the addition of any metal compound or methanol, the cell population reached $1.8 \times 10^8$ cells/ml. As shown in Table I, metal compounds were tested in the concentration range of $1 \times 10^{-2}$ to $1 \times 10^{-7}$ M. Each metal compound was tested for RD mutant induction at the highest concentration showing almost no growth inhibition (+++ in Table I), and at one order higher concentration, if only slight growth inhibition was recognized (++ in Table I).

### Effects of Methanol on the Induction of RD Mutants by Metal Ions

As shown in Table II, the frequency of spontaneous RD mutants in *S. cerevisiae* ATCC 26422 was 1.4% after 24-h incubation at 30 °C. Methanol did not show any mutagenic or growth-inhibitory effect on the yeast. At concentrations having no growth inhibitory effect, $ZnCl_2$, $HgCl_2$, $NiCl_2$, $CdCl_2$ and $MnCl_2$ induced RD mutants in the range of 1.8 to 2.2%. The addition of 4% methanol to the culture medium decreased the induction of RD mutants to the spontaneous level. This effect of methanol on the RD mutant induction was weaker in the case of $PbCl_2$ and $CuCl_2$. The induction of RD mutants by $CoCl_2$ was not affected by the addition of 4% methanol.

As shown in Table II, the growth of yeast was inhibited by higher concentrations of metal ions, and the induction rate of RD mutants by $Ni^{2+}$, $Mn^{2+}$ and $Cd^{2+}$ increased sharply. Both $CdCl_2$ ($10^{-6}$ M) and $MnCl_2$ ($10^{-2}$ M) were the most effective of all the metal ions tested and could induce 13.0 and 13.5% RD mutants, respectively. Except for the case of $Mn^{2+}$,

Table I. Effect of Metal Compounds on Yeast Growth

| Compound | Concentration (M) | | | | | |
|---|---|---|---|---|---|---|
| | $1 \times 10^{-7}$ | $1 \times 10^{-6}$ | $1 \times 10^{-5}$ | $1 \times 10^{-4}$ | $1 \times 10^{-3}$ | $1 \times 10^{-2}$ |
| $MnCl_2$ | | | | | +++ | ++ |
| $PbCl_2$ | | | | | +++ | ++ |
| $ZnCl_2$ | | | | | +++ | − |
| $CoCl_2$ | | | | +++ | ++ | + |
| $NiCl_2$ | | | | +++ | ++ | − |
| $CuCl_2$ | | | +++ | ++ | − | − |
| $HgCl_2$ | | | +++ | − | − | − |
| $CdCl_2$ | +++ | ++ | − | − | − | − |

Yeast cells were inoculated into Ogur's medium at $1 \times 10^5$ cells/ml and incubated at 30 °C with metal compounds. After a 24-h incubation, the medium was diluted appropriately and spread onto agar plates to give about 200 colonies. Colonies formed were counted and converted into per ml medium; +++ ($>10^8$ colonies), ++ ($10^8$—$10^7$ colonies), + ($10^7$—$10^5$ colonies) and − ($<10^5$ colonies).

NII-Electronic Library Service

TABLE II. Effects of Methanol on Growth and Induction of RD Mutants by Metal Compounds in Yeast

| Compound | Concn. (M) | 4% methanol | Cells per ml culture $\times 10^8$ | RD mutants (%) |
|---|---|---|---|---|
| None | | − | 1.8 | 1.4±0.8 |
| | | + | 2.0 | 0.9±0.3 |
| $ZnCl_2$ | $1\times10^{-3}$ | − | 1.3 | 2.1±1.1 |
| | | + | 1.6 | 1.6±1.5 |
| $HgCl_2$ | $1\times10^{-5}$ | − | 1.7 | 2.0±1.1 |
| | | + | 1.6 | 1.4±1.0 |
| $NiCl_2$ | $1\times10^{-4}$ | − | 1.4 | 1.8±0.6 |
| | | + | 1.8 | 1.4±1.1 |
| | $1\times10^{-3}$ | − | 0.7 | 7.2±4.6 |
| | | + | 1.6 | 0.9±0.6 |
| $CdCl_2$ | $1\times10^{-7}$ | − | 1.8 | 2.0±1.2 |
| | | + | 1.6 | 1.4±0.8 |
| | $1\times10^{-6}$ | − | 0.7 | 13.0±5.7 |
| | | + | 1.2 | 1.6±1.5 |
| $MnCl_2$ | $1\times10^{-3}$ | − | 1.6 | 2.2±1.5 |
| | | + | 1.7 | 1.5±1.4 |
| | $1\times10^{-2}$ | − | 0.5 | 13.5±5.0 |
| | | + | 0.03 | 60.7±14.2 |
| $PbCl_2$ | $1\times10^{-3}$ | − | 1.7 | 3.1±2.1 |
| | | + | 1.7 | 2.0±1.0 |
| | $1\times10^{-2}$ | − | 0.3 | 3.4±0.8 |
| | | + | 0.3 | 1.9±1.5 |
| $CuCl_2$ | $1\times10^{-5}$ | − | 1.8 | 2.2±1.1 |
| | | + | 1.7 | 1.9±0.6 |
| | $1\times10^{-4}$ | − | 0.3 | 2.7±2.2 |
| | | + | 0.5 | 1.9±1.7 |
| $CoCl_2$ | $1\times10^{-4}$ | − | 1.2 | 5.6±5.2 |
| | | + | 1.5 | 5.3±4.2 |
| | $1\times10^{-3}$ | − | 0.7 | 4.6±3.1 |
| | | + | 0.5 | 4.2±1.3 |

Yeast cells were inoculated into Ogur's medium at $1\times10^5$ cells/ml and incubated at 30 °C for 24 h with or without each metal compound and 4% methanol. Cells were diluted and spread onto Ogur's agar plates and incubated at 30 °C for 2 d. RD mutants were scored by the tetrazolium salt overlay method. Growth was expressed as colony-forming cells/ml culture. Results were the average of at least three independent experiments ± S.D.

TABLE III. Effects of Magnesium Chloride on Induction of RD Mutants by Manganese Chloride in Yeast

| Compounds ($1\times10^{-2}$ M) | | 4% methanol | Cells per ml culture $\times 10^8$ | RD mutants (%) |
|---|---|---|---|---|
| $MnCl_2$ | $MgCl_2$ | | | |
| + | − | − | 0.5 | 13.5±5.0 |
| + | − | + | 0.03 | 60.7±14.2 |
| − | + | − | 1.9 | 2.3±1.0 |
| − | + | + | 2.1 | 1.1±0.5 |
| + | + | − | 0.6 | 4.8±1.8 |
| + | + | + | 0.6 | 2.2±0.6 |

Details are the same as in Table II.

NII-Electronic Library Service

TABLE IV. Induction of RD Mutants of Yeast by Manganese or Cadmium Chloride in Magnesium Ion-Free Medium

| Compound | Concn. (M) | 4% methanol | Cells per ml culture $\times 10^8$ | RD mutants (%) |
|---|---|---|---|---|
| None | | − | 1.5 | 1.0±1.0 |
| | | + | 1.9 | 1.2±1.0 |
| $MnCl_2$ | $1\times10^{-3}$ | − | 0.3 | 16.8±4.0 |
| | | + | 0.03 | 63.8±5.8 |
| $CdCl_2$ | $1\times10^{-6}$ | − | 0.3 | 26.1±9.7 |
| | | + | 1.1 | 1.7±1.2 |

$MgSO_4\cdot7H_2O$ ($4\times10^{-3}$ M) in Ogur's medium was omitted. Other conditions were the same as in Table II.

methanol still repressed the RD mutant induction to the spontaneous level as found with lower concentration of metal ions. In contrast, methanol enhanced the RD mutant induction by $Mn^{2+}$ to 60.7%. The growth inhibition by higher concentration of metal ions such as $Ni^{2+}$, $Cd^{2+}$ and $Cu^{2+}$ was reversed to some extent by the addition of methanol, but that of $Mn^{2+}$ was greatly enhanced.

**Effect of Magnesium Chloride on the Induction of RD Mutants by Manganese Chloride**

As shown in Table III, $1\times10^{-2}$ M $Mg^{2+}$ induced only 2.3% RD mutants and did not inhibit the growth of yeast cells. The induction of RD mutants by $Mn^{2+}$ decreased from 13.5 to 4.8% on the addition of $Mg^{2+}$. The effect of $Mg^{2+}$ was remarkable in the presence of 4% methanol, and the induction of RD mutants decreased from 60.7 to 2.2%. The growth inhibitory effect of $Mn^{2+}$ was also reversed by the addition of $Mg^{2+}$.

**The Induction of RD Mutants by Manganese and Cadmium Chlorides in Magnesium Ion-Free Medium**

Since Ogur's medium contained 0.1% ($4\times10^{-3}$ M) $MgSO_4\cdot7H_2O$, this constituent was omitted to examine the effect of $Mg^{2+}$ on the induction of RD mutants. Both $Mn^{2+}$ and $Cd^{2+}$ were tested because of their high mutagenicity. In this medium, $1\times10^{-2}$ M $MnCl_2$ inhibited the cell growth almost completely (data not shown). As shown in Table IV, $1\times10^{-3}$ M $MnCl_2$, which was only weakly mutagenic in the complete Ogur's medium, induced 16.8% RD mutants. Methanol enhanced the induction of RD mutants considerably. On the other hand, the RD mutation induced by $Cd^{2+}$ in the $Mg^{2+}$-free medium did not show any marked change compared with that in complete Ogur's medium.

## Discussion

Though the mechanisms are still obscure, many metal ions have been reported to induce cytoplasmic RD mutants in yeast.[5-8] In this study we have shown that methanol decreases the induction rate of RD mutants by most of the metal ions tested. This methanol effect was already reported in connection with the induction of RD mutants by aromatic alcohols[3] and acriflavine.[4] These facts suggest that methanol may affect the common process(es) in the induction of RD mutants, *i.e.* the uptake of mutagens into yeast cells and/or mitochondria, and expression of the RD phenotype[11] after the action of mutagens. In this respect, we have reported that methanol partially inhibits uptake of acriflavine by yeast cells.[4] The recovery of the cell growth by methanol may also depend on the methanol-induced prevention of metal ion uptake or accumulation. Methanol apparently does not affect the expression of the RD phenotype, since methanol enhanced the RD mutation induced by $Mn^{2+}$.

NII-Electronic Library Service

The marked enhancement of $Mn^{2+}$-induced RD mutation by methanol suggested that $Mn^{2+}$ may induce RD mutants by a mechanism different from that of other metal ions. Putrament *et al.*[7] have reported that the induction of RD mutants by $Mn^{2+}$ results from a decrease in the fidelity of mitochondrial deoxyribonucleic acid (mitDNA) synthesis. They[12] concluded that $Mn^{2+}$ interacts with yeast mitDNA polymerase which has a definite preference for $Mg^{2+}$.[13] Methanol is considered to help the action of $Mn^{2+}$ as an error-producing factor during the replication of mitDNA. For instance, if methanol stimulates the induction of mitDNA polymerase, the amount of abnormal mitDNA polymerase in which $Mg^{2+}$ is replaced by $Mn^{2+}$ may increase and the fidelity of mitDNA synthesis may be affected. Further experiments are required to elucidate the mechanism of methanol effect on the induction of RD mutants by $Mn^{2+}$.

The induction of RD mutants by $Mn^{2+}$ is prevented almost completely by the addition of $Mg^{2+}$ (Table III). The same effect of $Mg^{2+}$ on the RD mutant induction by $Mn^{2+}$ was previously reported.[7,8] Fuhrmann and Rothstein[14] and Rothstein *et al.*[15] have reported a competition between $Mn^{2+}$ and $Mg^{2+}$ transport into yeast cells. $Mg^{2+}$ was taken up preferentially over $Mn^{2+}$ and can totally inhibit $Mn^{2+}$ uptake when added at the same concentration.[15] This inhibition of $Mn^{2+}$ uptake by $Mg^{2+}$ is in agreement with the result presented in this report that $Mg^{2+}$ antagonizes $Mn^{2+}$ in terms of cell growth. The growth and the RD mutant induction in $Mg^{2+}$-free medium (Table IV) also show an inhibitory effect of $Mg^{2+}$ on $Mn^{2+}$ transportation but not on $Cd^{2+}$ transportation into yeast cells. Detailed studies on the concentration dependence of the methanol effects and on the correlation between the methanol effects and cell growth are in progress.

**Acknowledgement** The authors thank Misses S. Iwahashi, M. Matsuura, K. Nishi and Y. Yamada, and Messrs S. Hatano, Y. Kugiyama and M. Suemasu for their technical assistance.

## References

1) N. Kato and Y. Tani, *Fermentat. Industry*, **40**, 128 (1980).
2) *a*) K. Yamashiro, H. Nishihara, M. Shimoide, Y. Tani, and S. Fukui, *J. Fermentat. Technol.*, **48**, 307 (1970); *b*) K. Ohta, K. Supanwong, and S. Hayashida, *ibid.*, **59**, 435 (1981); *c*) H. Hamada, Y. Shimotakahara, Y. Kurihara, and M. Kojima, *ibid.*, **62**, 81 (1984).
3) M. Kojima, A. Matsuo, H. Hamada, and T. Morita, *Hakkokogaku*, **57**, 315 (1979).
4) H. Hamada, M. Yamashita, M. Kojima, and T. Morita, *Chem. Pharm. Bull.*, **32**, 623 (1984).
5) C. C. Lindegren and G. Lindegren, *Mutat. Res.*, **21**, 315 (1973).
6) C. C. Lindegren, S. Nagai, and H. Nagai, *Nature* (London), **182**, 446 (1958).
7) A. Putrament, H. Baranowska, and W. Prazmo, *Molec. Gen. Genet.*, **126**, 357 (1973).
8) C. Donnini, P. P. Puglisi, and N. Marmiroli, *Molec. Gen. Genet.*, **190**, 504 (1983).
9) M. Ogur and S. John, *J. Bacteriol.*, **73**, 500 (1956).
10) S. Nagai, *Science*, **130**, 1188 (1959).
11) C. Gaillard, F. Strauss, and G. Bernardi, *Nature* (London), **283**, 218 (1980).
12) A. Putrament, H. Baranowska, A. Ejchart, and W. Jachymczk, *Molec. Gen. Genet.*, **151**, 69 (1977).
13) E. Wintersberger, *Biochem. Biophys. Res. Commun.*, **25**, 1 (1966).
14) G.-F. Fuhrmann and A. Rothstein, *Biochim. Biophys. Acta*, **163**, 325 (1968).
15) A. Rothstein, A. Hayes, D. Jennings, and D. Hooper, *J. Gen. Physiol.*, **41**, 585 (1958).

NII-Electronic Library Service