Biotechnol. Bioprocess Eng. **1998**, 3, 15-18

Anaerobic Respiration of Superoxide Dismutase-Deficient *Saccharomyces cerevisiae* under Oxidative Stress

Sun Mi Lee and Doo Hyun Nam*

College of Pharmacy, Yeungnam University, Kyongsan 712-749, Korea

The ethanol productivity of superoxide dismutase (SOD)-deficient mutants of *Saccharomyces cerevisiae* was examined under the oxidative stress by Paraquat. It was observed that MnSOD-deficient mutant of *S. cerevisiae* had higher ethanol productivity than wild type or CuZnSOD-deficient yeast both in aerobic and in anaerobic culture condition. Pyruvate dehydrogenase activity decreased by 35% and alcohol dehydrogenase activity increased by 32% were observed in MnSOD-deficient yeast grown aerobically. When generating oxygen radicals by Paraquat, the ethanol productivity was increased by 40% in CuZnSOD-deficient or wild strain, resulting from increased activity of alcohol dehydrogenase and decreased activity of pyruvate dehydrogenase. However, the addition of ascorbic acid with Paraquat returned the enzyme activities at the level of control. These results imply that SOD-deficiency in yeast strains may cause the metabolic flux to shift into anaerobic ethanol fermentation in order to avoid their oxidative damages by Paraquat.

Key words: ethanol productivity, anaerobic respiration, metabolic shift, *Saccharomyces cerevisiae*, oxidative stress, superoxide dismutase, Paraquat

INTRODUCTION

Most living organisms use oxygen through aerobic aeration for maintenance and growth. However, a little amount of oxygen radicals such as superoxide anion, hydroxyl radical and hydrogen peroxide produced by this process can cause an undesirable damage. In order to rescue from oxidative damages, superoxide dismutase (SOD), catalase, glutathione peroxidase and myeloperoxidase also used as oxygen radical scavenging systems [1].

Yeast, as the simplest eucaryotic organism, has two different SODs; one is CuZnSOD having Cu^{2+} and Zn^{2+} at an active site in the cytoplasmic fraction, the other is MnSOD having Mn^{2+} in the mitochondrial part, as likely as higher eucaryotes [2]. In addition, yeast cells are thought to be a good model system in oxygen toxicity experiments because they are capable of growing both in aerobic and in anaerobic conditions. Thus, the change of cellular activity of SOD and catalase in *Saccharomyces cerevisiae* was intensively investigated during cultivation in chemostat by Hassan's group [3-4].

Using SOD-deficient yeast cells, we have already reported the response against oxidative stresses induced by Paraquat and copper ion, in order to elucidate the role of SOD in cellular metabolism and oxygen radical toxicity [5-6]. In this study, we examined the ethanol productivity and change of the metabolic flux in SOD-deficient yeast cells, especially under oxidative stress generated by Paraquat.

MATERIALS AND METHODS

Microorganisms and Culture Condition

Four yeast strains were used throughout this work; *Saccharomyces cerevisiae* EG103 (*MAT leu2-3,112 his 3Δ1 trp1-289a ura3-52 gal*$^+$) as wild type, *S. cerevisiae* EG110 (same except *sod2Δ::TRP1*) lacking mitochondrial MnSOD, *S. cerevisiae* EG118 (same except *sod1ΔA::URA*) lacking cytoplasmic CuZnSOD, and *S. cerevisiae* EG133 (same except *sod1ΔA::URA* and *sod 2Δ::TRP1*) deficient of both SOD enzymes, all of which were kindly sent by Dr. Edith B. Gralla at University of California, Los Angeles, CA, U.S.A [7]. The yeast strains were grown on YPD media which consists of 1% yeast extract, 2% peptone, and 2% glucose, for 16 h at 30°C with rotary shaking at 150 rpm. For anaerobic cultivation, the tightly capped Erlenmeyer flasks with a side arm of spectrophotometric cell were employed. To keep the culture broth anaerobic, 0.03% sodium thioglycollate was supplemented in YPD medium with 1 ppm of resazurin as an indicator. Paraquat, 0.01 mM was added into culture broth, in case of generating the oxygen toxicity. If necessary, 0.1 mM of ascorbic acid was supplemented into culture broth as an antioxidant agent.

Analytical Procedures

The yeast cells were harvested by centrifugation at 5,000× g after 16 hr-cultivation, washed twice and resuspended in 50 mM phosphate buffer (pH 7.5) containing 0.1 mM ethylenediamine tetraacetate (EDTA), and then disrupted by ultrasonication for 5 min (XL 2010 Ultrasonic Processor, Heat Systems, NY, USA). The supernatant obtained by centrifuging at 27,000× g was used as crude enzyme sources for alcohol dehydrogenase and pyruvate dehydrogenase. The activity of pyruvate dehydrogenase was measured by the method of Hinman and Blass [8] using pyruvate and CoA-SH as substrates, and the activity of alcohol dehydrogenase was determined following the procedure of

* Corresponding author
Tel: 82-53-810-2825 Fax: 82-53-811-3871
e-mail: dhnam@ynucc.yeungnam.ac.kr

Bernt and Gutmann [9] using ethanol as substrate. Both enzyme activities were expressed as the produced amounts of NADH during reactions per min per mg protein. The cell mass of yeast was measured spectrophotometrically at 600 nm, and converted to dry cell weight using standard curve. The protein content in cell lysates was analyzed by Folin-Lowry method [10] using bovine serum albumin as a standard.

The amount of ethanol produced in culture broth was assayed by gas chromatography (Hewlett Packard, CA, USA) using a polyethylene glycol-TPA modified capillary column (25m × 0.32 mm × 0.52 μm) and flamed ionization detector (240°C). One μl of sample was injected into 220°C port (split ratio=1.99), and passed through 200°C column by mixed carrier gas of air: H_2: N_2 (10:1:0.76).

All the results were plotted as the mean values of more than 3 separate experimental data.

RESULTS AND DISCUSSION

Ethanol Productivity of SOD-deficient Yeast Strains under Oxidative Stress

When the ethanol productivities of SOD-deficient yeast strains were compared, the MnSOD-deficient yeast mutant (EG110) had 75(90% higher ethanol productivity than CuZnSOD-deficient one (EG118) or wild strain (EG103) during aerobic cultivation, and 55(65% higher at anaerobic culture condition, as shown in Fig. 1. When exposed to Paraquat, one of well-known free radical generating agents, it was observed that MnSOD-deficient yeast produced 30(40% more ethanol than CuZnSOD-deficient strain or wild strain under aerobic culture conditions, and more than 40% when cultivated anaerobically. From the above results, it may be assumed that yeast cells may shift their intracellular metabolism into anaerobic ethanol fermentation in order to avoid oxidative damages generated by Paraquat.

Especially in MnSOD-deficient yeast strain, the oxidative respiration in mitochondrion can be repressed under aerobic condition due to its absence of mitochondrial scavenging system for oxygen radical, and anaerobic respiration in cytoplasm can be activated in place. As seen in Fig. 1, Paraquat increased ethanol productivity by only 5(7% in MnSOD-deficient yeast, but 40% higher ethanol productivity occurred in CuZnSOD-deficient strain and wild strain cultivated aerobically. It may be due to Paraquat producing oxygen radicals mainly in mitochondrial fraction, as previously reported [3]; Paraquat was not affecting mitochondrial respiration any further in MnSOD-deficient yeast, which were already inhibited by aerobic cultivation.

Differently, CuZnSOD-deficient strain could activate the cytoplasmic anaerobic respiration only when free radicals were generated in mitochondria by Paraquat. To confirm this assumption, the effect of ascorbic acid, an antioxidant, on ethanol productivity was examined. The supplementation of ascorbic acid in culture broth could reduce the ethanol productivity to the level of 105(115% when cultivated aerobically and to 85(95% level at anaerobic condition, compared to the values before exposing to Paraquat (Fig. 1). This result strongly support that the anaerobic respiration could be activated in the cytoplasm of yeast strains by aerobic cultivation, and that this effect was much increased by oxidative stress of Paraquat.

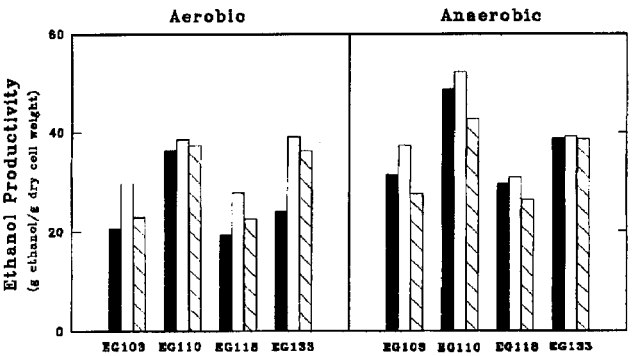

Fig. 1. Ethanol productivity of yeast strains cultured aerobically or anaerobically in the presence of Paraquat or Paraquat/ascorbic acid.
S. cerevisiae EG103 (wild strain), EG110 (MnSOD-deficient mutant), EG118 (CuZnSOD-deficient mutant), and EG133 (mutant lacking both SODs) were cultivated aerobically or anaerobically without Paraquat (■), with supplementing 0.01 mM Paraquat (□), or with simultaneous supplementing 0.01 mM Paraquat and 0.1 mM ascorbic acid (▨).

Metabolic Pattern of SOD-deficient Yeast Strains under Oxidative Stress

In order to ascertain the metabolic shift to anaerobic respiration in SOD-deficient yeast strains, the intracellular activity of pyruvate dehydrogenase involved in aerobic respiration and that of alcohol dehydrogenase in anaerobic ethanol fermentation were measured and compared. Under aerobic culture condition, all the yeast strains showed 20(50% higher activity of pyruvate dehydrogenase (Fig. 2) and nearly 30% lower activity of alcohol dehydrogenase (Fig. 3), compared to anaerobic cultivation. Especially when MnSOD-deficient yeast was grown aerobically, 35% decrease of pyruvate dehydrogenase activity and 32% increase of alcohol dehydrogenase activity were observed, compared to wild strain. Under anaerobic conditions, this mutant strain showed 18% lower activity of py-

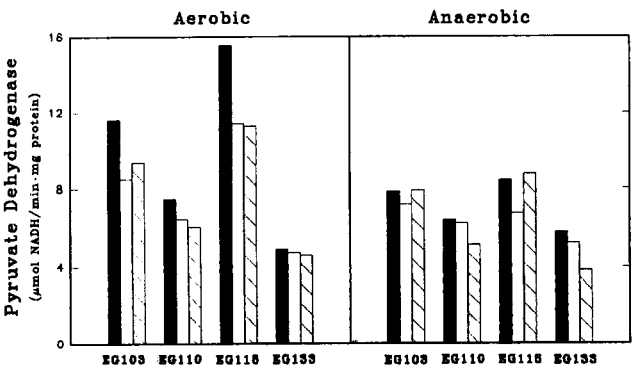

Fig. 2. Total cellular activities of pyruvate dehydrogenase in yeast strains cultured aerobically or anaerobically in the presence of Paraquat or Paraquat/ascorbic acid.
S. cerevisiae EG103 (wild strain), EG110 (MnSOD-deficient mutant), EG118 (CuZnSOD-deficient mutant), and EG133(mutant lacking both SODs) were cultivated aerobically or anaerobically without Paraquat (■), with supplementing 0.01 mM Paraquat (□), or with simultaneous supplementing 0.01 mM Paraquat and 0.1 mM ascorbic acid (▨).

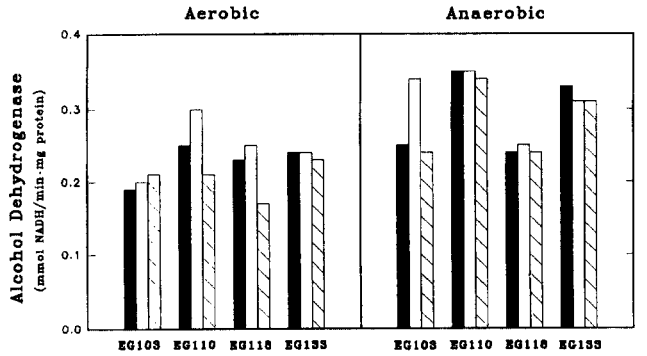

Fig. 3. Total cellular activities of alcohol dehydrogenase in yeast strains cultured aerobically or anaerobically in the presence of Paraquat or Paraquat/ascorbic acid.
S. cerevisiae EG103 (wild strain), EG110 (MnSOD-deficient mutant), EG118 (CuZnSOD-deficient mutant), and EG133(mutant lacking both SODs) were cultivated aerobically or anaerobically without Paraquat (■), with supplementing 0.01 mM Paraquat (□), or with simultaneous supplementing 0.01 mM Paraquat and 0.1 mM ascorbic acid (▨).

ruvate dehydrogenase activity and 40% higher activity of alcohol dehydrogenase than wild yeast, which strongly suggests that the deficiency of mitochondrial MnSOD may increased ethanol production.

CuZnSOD-deficient yeast under aerobic condition had 35% higher activity of pyruvate dehydrogenase and 20% higher activity of alcohol dehydrogenase than wild type. However, not so remarkable change in the activities of these enzymes was not found in this strain at anaerobic condition. This can be explained by the deficiency of cytoplasmic CuZnSOD driving the cell into the aerobic mitochondrial respiration rather than ethanol fermentation due to oxygen radicals in cytoplasm, but any metabolic change could not be accompanied at anaerobic state.

Paraquat generating oxygen radicals mostly in mitochondria was verified by the decreasing pattern of pyruvate dehydrogenase activity. When inducing oxygen stress by Paraquat, 30% decrease in wild strain and CuZnSOD-deficient mutant and 15% in MnSOD-deficient one were observed when cultivated aerobically (Fig. 2). At anaerobic condition, 10% of pyruvate dehydrogenase activity in wild strain and 20% in CuZnSOD-deficient mutant were decreased by Paraquat, but any remarkable change in MnSOD-deficient yeast was not noticed. This result shows that the free oxygen radicals by Paraquat could inhibit aerobic mitochondrial respiration pathway through the pyruvate dehydrogenase repression in yeast strains. Thus the pyruvate dehydrogenase activity of MnSOD-deficient strain was less affected by exposure to Paraquat. Even though the supplementation of ascorbic acid with Paraquat in culture media did not significantly affect the activity of pyruvate dehydrogenase in all the yeast strains under aerobic condition, ascorbic acid completely recovered this enzyme activity in wild strain and CuZnSOD-deficient mutant strain at the same level as control when cultivated anaerobically. It may be attributed that ascorbic acid could successfully keep the mitochondrial respiration of CuZnSOD-deficient yeast from oxygen damage by Paraquat.

In case of alcohol dehydrogenase, 20% higher increment in cellular activity was observed in MnSOD-deficient mutant exposed to Paraquat under aerobic condition, whereas its activity was increased by 5% in wild strain or 10% in CuZnSOD-deficient one. Comparatively, the enzyme activity in wild strain was increased as much as 35% at anaerobic state, although MnSOD-deficient and CuZnSOD-deficient strains were detected. From this, it can be assumed that mitochondrial free radicals by Paraquat under aerobic condition could increase the activity of cytoplamic alcohol dehydrogenase for anaerobic respiration in yeast strains. In addition, all the yeast strains reduced their cellular activity of alcohol dehydrogenase to nearly the same level as control when ascorbic acid was supplemented simultaneously with Paraquat in culture media. This can be also explained by oxygen radicals in mitochondria by Paraquat, enhancing the activity of alcohol dehydrogenase in cytoplasm.

In conclusion, Paraquat can generate oxygen radicals in mitochondria, finally to cause the inhibition of aerobic respiration simultaneously with the activation of anaerobic respiration in cytoplasm. Especially in MnSOD-deficient strain, the oxidative stress in mitochondria by aerobic cultivation or by exposure to Paraquat may cause the activation of anaerobic fermentation in cytoplasm instead of mitochondrial respiration, which results in higher ethanol productivity. Nevertheless, CuZnSOD-deficient mutant lacking cytoplasmic free radical scavenging system, cannot help enhancing the mitochondrial respiration even under oxidative stress.

Acknowledgement This work was kindly supported by Korean Ministry of Education Biochemical Engineering Program (1995), and partly by Yeungnam University for the academic research promotion (1995). The authors would like to express their deep thanks to Dr. Edith Butler Gralla (University of California, Los Angeles) for his kindness to provide yeast strains.

REFERENCES

[1] Deby, C. and R. Goutier (1990) New perspectives on the biochemistry of superoxide anion and the efficiency of superoxide dismutases. *Biochem. Pharmacol.* 39: 399-405.
[2] Galiazzo, F., M. T. Carri, M. R. Ciriolo, and G. Rotilio (1994) Superoxide dismutase in *Saccharomyces cerevisiae*. *Mycol. Ser.* 11: 361-390.
[3] Lee, F.-J. S. and H. M. Hassan (1986) Biosynthesis of superoxide dismutase and catalase in *Saccharomyces cerevisiae*: effects of oxygen and cytochrome c deficiency. *J. Ind. Microbiol.* 1: 187-193.
[4] Lee, F.-J. S. and H. M. Hassan (1987) Biosynthesis of superoxide dismutase and catalase in chemostat culture of *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol.* 26: 531-536.
[5] Kim, J. M., D. H. Nam, C. S. Yong, and K. Huh (1995) Oxygen toxicityof superoxide dismutase-deficient *Saccharomyces cerevisiae* by paraquat. *Kor. J. Biotechnol. Bioeng.* 10: 561-567.
[6] Lee, J. K., J. M. Kim, S. W. Kim, D. H. Nam, C. S. Yong, and K. Huh (1996) *Arch. Pharm. Res.* 19: 178-182.
[7] Liu, X. F., I. Elashvili, E. B. Gralla, J. S. Valentine, P. Lapinskas, and V. C. Culotta (1992) Yeast lacking superoxide dismutase. Isolation of genetic suppressors. *J. Biol. Chem.* 267: 18298-18302.
[8] Hinman, L. M. and Blass, J. P. (1981) An NADH-linked spectrophotometric assay for pyruvate

dehydrogenase complex in crude tissue homogenates. *J. Biol. Chem.* 256: 6583-6586.

[9] Bernt, E. and I. Gutmann (1974) Ethanol-determination with alcohol dehydrogenase and NAD. p.1499-1502, In: H.U. Bergmeyer (ed) *Methods of Enzymatic Analysis*, vol.3, Verlag Chemie, Weinheim.

[10] Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265-275.