Volume 315, number 2, 197–200 FEBS 11969 January 1993

Regulation of Cu,Zn- and Mn-superoxide dismutase transcription in *Saccharomyces cerevisiae*

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Received 11 November 1992

The regulation of Cu,Zn- and Mn-superoxide dismutases (SOD) was investigated by Northern blotting and gene fusions of *SOD1* and *SOD2* promoters with the β-galactosidase reporter gene. Cu,ZnSOD expression was increased 3-fold under glucose derepressing conditions, and decreased 4- to 6-fold by oxygen or heme deficiency. MnSOD expression was increased 5-fold by glucose derepression, and decreased 8- to 10-fold by anaerobiosis and 4- to 5-fold by heme deficiency. Induction by paraquat was modest, about 50% for *SOD1* and 100% for *SOD2*; it was apparently independent of the respiratory chain function.

Superoxide dismutase; Paraquat; Oxygen and heme; Transcriptional regulation; *Saccharomyces cerevisiae*

1. INTRODUCTION

Superoxide anion radicals (O_2^-) are potentially toxic byproducts of oxidative metabolism, and all aerobic organisms have evolved enzymic mechanisms to overcome oxygen radical-mediated toxicity. The superoxide dismutases are metalloenzymes which convert superoxide radicals to hydrogen peroxide and oxygen. In *E. coli* [1] and plants [2], their synthesis is regulated in response to oxidative stresses. The yeast *S. cerevisiae* contains two superoxide dismutases. The manganese-superoxide dismutase (MnSOD) encoded by the gene *SOD2* is located in the mitochondrial matrix [3], while the copper,zinc-superoxide dismutase (Cu,ZnSOD) encoded by the gene *SOD1* is in the cytosol [4]. Several reports indicate that the two enzymes are regulated in yeast. The activity or steady-state level of immunodetectable MnSOD is decreased under conditions of oxygen and heme deficiency and by glucose [5,6]. The observations that glucose and anaerobiosis also diminish the steady-state level of mRNA indicate that these regulations operate at the transcriptional level [3,7,8]. Both the activity of Cu,ZnSOD and the amount of enzyme protein are depressed under anaerobic conditions and part of the enzyme is in the form of an apoprotein which can be reactivated by the addition of copper [9]. Copper also partially controls the transcription of *SOD1* via the copper-dependent *trans*-activator *ACE1* gene product [10,11]. Lastly, both SOD activities are increased by the herbicide paraquat (methyl viologen), a redox-cycling compound used to increase the intracellular flux of O_2^- [12]. However, these results taken together do not provide a clear picture of the regulation of both SOD, primarily because of the differences in the experimental approaches used to estimate the expression of *SOD1* and *SOD2* genes. The present study analyzes the transcriptional activity of the *SOD* genes in response to carbon source, growth phase, oxygen, heme and paraquat, all in the same genetic background.

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2. MATERIALS AND METHODS

2.1. *Construction of the promoter-LacZ fusions*

Episomal and integrative plasmids carrying the *LacZ* fusions are based on the plasmids described in [13]. The *SOD1–LacZ* fusion was constructed by inserting upstream of the promoterless *LacZ* gene of YEp357 and YIp357 a polymerase chain reaction-amplified DNA fragment containing 410 nucleotides of the 5′ non-coding region and 105 codons of the amino-terminal coding sequence of *SOD1* [4]. The *SOD2–LacZ* fusion contained 558 nucleotides of the 5′ non-coding region and 6 codons of the amino-terminal coding region of *SOD2* [3] in YEp357R and YIp357R. The *HEM13–LacZ* fusion was obtained by inserting 1076 nucleotides of the 5′ non-coding region and 33 codons of the amino-terminal coding region of *HEM13* (coproporphyrinogen oxidase [14]) in YEp357. The plasmid pLG669-Z, containing the promoter and one codon of *CYC1* (iso-1-cytochrome *c*) in frame with *LacZ*, was used as the *CYC1–LacZ* fusion [15].

2.2. *Yeast strains and growth conditions*

All strains used in this work were derived from the strain S150-2B (*Mata leu2-3,112 ura3-52 trp1-289 his3 1*). The heme-deficient derivative strains *hem1Δ* or *hem15Δ* were obtained by replacing the *HEM1* gene (encoding 5-aminolevulinic acid synthase) or the *HEM15* gene (encoding ferrochelatase) with deleted null alleles (R. Labbe-Bois, unpublished). The episomal plasmids carrying the promoter-*LacZ* fusions were transformed into strain S150-2B (*HEM15* or *hem15Δ*) by

the lithium acetate method [16]. The integrative plasmid carrying the *SOD2–LacZ* fusion was integrated at the chromosomal *SOD2* locus after linearization with *Nhe*I. Correct integration was confirmed by Southern hybridization analysis. For unknown reasons, we did not succeed in integrating the *SOD1–LacZ* fusion plasmid at the *SOD1* locus. Respiratory-deficient (rho$^-$) derivatives were obtained after ethidium bromide mutagenesis [17].

Yeast cells were grown at 30°C with vigorous agitation. Special air-tight flasks were used for anaerobic growth [18]. Complete and minimal media [17] contained 2% glucose or 2% ethanol + 2% glycerol. Tween 80 (0.2%) and ergosterol (30 mg/l) were added for culturing heme-deficient cells and for anaerobic growth. Cells were collected in early logarithmic-growth phase (A_{600nm} = 1 and 0.1 for cultures in complete and minimal media, respectively).

2.3. *DNA and RNA manipulations*

All manipulations were performed according to standard protocols [17,19] except for the preparation of total yeast RNA [20]. Northern blot analysis of total RNA (25 μg) size-fractionated on formaldehyde/agarose gels was performed with the following probes radiolabeled by random priming: a 1-kb PCR-amplified DNA fragment encompassing the entire *SOD1* gene; a 1.8-kb *Bam*HI–*Sma*I fragment of *SOD2*; a 1.1-kb *Xho*I–*Hin*dIII fragment of the actin gene *ACT1* used to monitor RNA loading.

2.4. *β-galactosidase assay*

β-galactosidase activity was measured [15] on whole cells permeabilized with SDS and chloroform. The activity is expressed as Miller units (A_{420nm}/min/A_{600nm} of cells × 1000). Assays were run in duplicate for at least two independent transformants for each plasmid. The data reported are the average of the results obtained with a deviation of ≤20%.

3. RESULTS AND DISCUSSION

3.1. *Effects of carbon source, growth phase and heat shock*

The expression of *SOD1–LacZ* and *SOD2–LacZ* and the steady-state levels of *SOD1* and *SOD2* mRNAs were higher in cells grown under derepressing conditions, with glycerol + ethanol, than in cells grown in glucose (2 or 10%). The difference was approx. 3-fold for *SOD1* and 5-fold for *SOD2* (Table I, Fig. 1). This is in agreement with the previously reported repression of *SOD2* by glucose [3,6–8], and correlates with the glucose-induced 2- to 3-fold decrease in the amount of Cu,ZnSOD immunoreactive protein and activity [9]. In contrast, the amount of Cu,ZnSOD immunoreactive protein has been reported to be enhanced 50% under glucose-repressing conditions [6]. Our results clearly show that Cu,ZnSOD transcription is repressed by glucose. The expression of *SOD2–LacZ* is about 15% of that of *SOD1–LacZ*, which is consistent with reports that MnSOD activity accounts for 5 to 15% of the total SOD activity [12,21,22].

The expression of both *SOD–LacZ* increased 50–60% during exponential growth on glucose (Fig. 2). A similar but smaller (25–40%) increase was also observed in cells growing on ethanol and in rho$^-$ cells growing on glucose, suggesting that this increase was not due to glucose derepression. No further significant increase in *SOD2–LacZ* activity occurred when the cells entered the stationary-growth phase, while the activity of *SOD1–LacZ* fell to very low values (Fig. 2). Cells harvested 5 h after the end of the exponential-growth phase contained almost normal amounts of *SOD2* mRNA, while the *SOD1* and *ACT1* mRNAs were barely detectable (Fig. 1). These results indicate that the transcription of neither of the *SOD* genes is induced when the cells enter a resting state due to nutrient limitation, in contrast to the behaviour of the *CTT1* (catalase T) and *CYC7* (iso-2-cytochrome *c*) genes [23,24]. Their lack of response to heat shock, which induces *CTT1* and *CYC7* transcription [24,25], is further evidence that the two SODs do not behave as stress proteins. Neither the expression of the *SOD–LacZ* fusions nor the level of the SOD genes mRNAs was increased 15, 30 or 60 min after a shift from 23 to 39°C.

We also tested the effect of oleate on the expression of *SOD1–LacZ*, since Cu,ZnSOD is present in the peroxisomes of human fibroblasts, hepatoma cells and rat liver [26,27], and oleate induces the proliferation of peroxisomes and the activities of peroxisomal enzymes in yeast cells [28]. The expression of *SOD1–LacZ* did not increase during the 8 h following the addition of oleate to cells growing on ethanol medium. This suggests that yeast Cu,ZnSOD does not behave like a peroxisomal enzyme and is probably not translocated to the peroxisomes. This was confirmed by the absence of Cu,ZnSOD activity in induced purified peroxisomes (M. Skoneczny and J. Rytka, personal communication).

Table I

Effects of glucose, anaerobiosis and heme-deficiency on the expression of *SOD1*, *SOD2*, *CYC1* and *HEM13* genes

Growth conditions	β-Galactosidase activity			
	SOD1–LacZ	*SOD2–LacZ*	*CYC1–LacZ*	*HEM13–LacZ*
Ethanol + glycerol	3350	750	1600	nd[a]
Glucose	980	163	550	40
Anaerobiosis	238	19	2	1460
Heme-deficiency	175	48	2	1520

Strain S150-2B and its heme-deficient derivative (*hem15Δ*) were transformed with the episomal plasmids carrying the different *LacZ*-fusions. Cells were grown in minimal selective medium (−ura) and harvested in early logarithmic phase (A_{600nm} = 0.1). [a] Not determined.

3.2. *Effects of oxygen and heme deficiency*

The expression of *SOD1* and *SOD2* genes was diminished in cells growing anaerobically and in heme-deficient cells (Table I, Fig. 1). The expression of *CYC1–LacZ* and *HEM13–LacZ*, regulated by oxygen and heme in opposite fashion, was analyzed as controls. Anaerobiosis caused a 4- to 6-fold decrease in *SOD1* expression, in agreement with the 4-fold anaerobiosis-induced decrease in the Cu,ZnSOD activity and immunoreactive protein [9]. A similar decrease under heme deficiency has not been reported previously. Thus, *SOD1* belongs to the large family of genes positively regulated by oxygen and heme [29].

SOD2 expression was reduced 8- to 10-fold under anaerobic conditions, as was the level of MnSOD immunodetectable protein [5]. Curiously, heme deficiency led to a smaller effect, as already noted for the amount of immunoreactive protein [5]. This might suggest that oxygen and heme regulate *SOD2* by different mechanism(s).

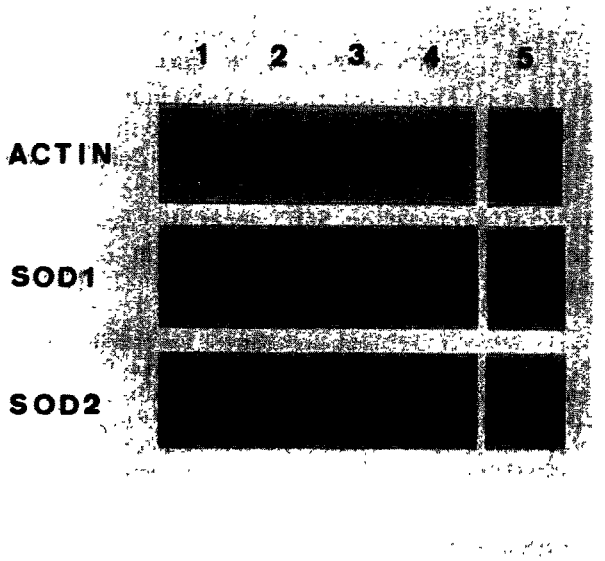

Fig. 1. Effects of glucose, oxygen- and heme deficiency on *SOD1* and *SOD2* mRNA levels. Strain S150-2B was grown aerobically in complete medium with ethanol + glycerol (lane 1) or 2% glucose (lanes 2, 5), or anaerobically with 2% glucose (lane 3). Its *hem1Δ* derivative was grown with 2% glucose (lane 4). Cells were harvested in early log phase (A_{600nm} = 1) (lanes 1–4) or in stationary phase (A_{600nm} = 10) (lane 5).

3.3. *Effect of paraquat*

Previous studies [12] have shown that the activities of both SOD almost double in cells growing in presence of paraquat. The present study shows that this effect operates at the transcriptional level. Induction was maximal with 2.5 mM paraquat at the end of the logarithmic-growth phase on glucose: about 50% for *SOD1* and 100% for *SOD2* (Fig. 2). Paraquat (2.5 mM) had a weaker effect on cells growing in ethanol + glycerol; a 20–25% increase in *SOD1–LacZ* and a 10–15% increase in *SOD2–LacZ* were observed 5 h after the addition of

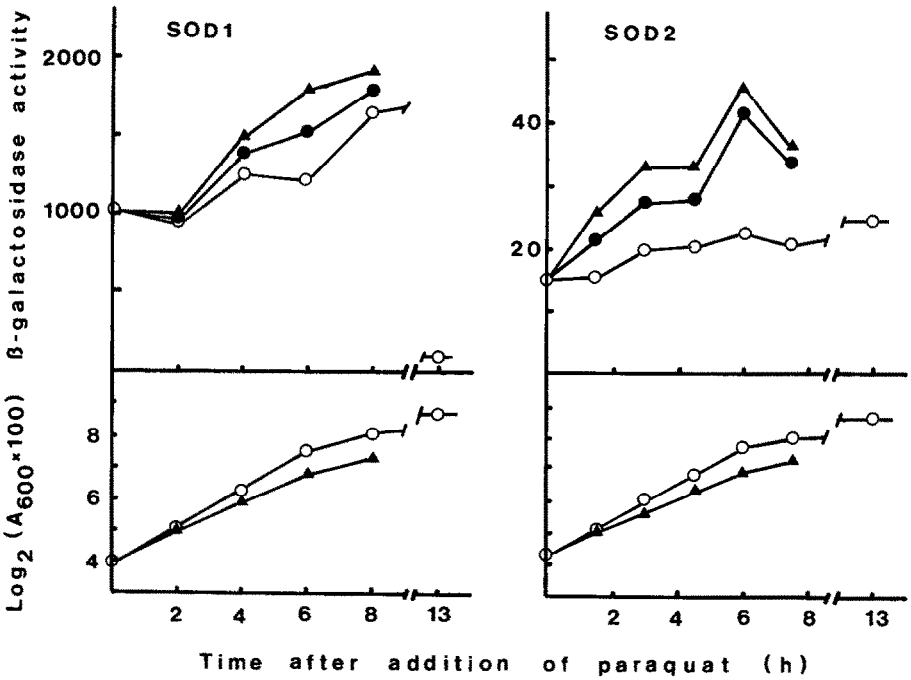

Fig. 2. Effect of paraquat on the expression of *SOD1* and *SOD2* genes. The *SOD1–LacZ* fusion gene was carried on an episomal plasmid. The *SOD2–LacZ* fusion gene was integrated at the *SOD2* locus. Cells were grown in minimal selective medium (−ura) to early log phase and then incubated without (○) or with 0.5 (●) or 2.5 (▲) mM paraquat. Samples were taken at the indicated intervals and β-galactosidase activity and A_{600nm} were measured. The values reported are for a single experiment. Two other identical experiments gave similar results.

paraquat, before the arrest of growth due to the high paraquat toxicity under these conditions [30]. *SOD2–LacZ* expression was reduced 30–40% in cells made rho$^-$ after ethidium treatment and was induced 40–45% by paraquat (2.5 mM) after 3 to 5 h. We thus conclude that the effect of paraquat on MnSOD does not seem to depend on the respiratory chain function. In fact, the effects of paraquat on SOD genes in yeast are very discrete, compared to those reported in *E. coli* and plants [1,2].

Acknowledgements: We thank L.A. Grivell for providing plasmid pFL1-41B containing the *SOD2* gene and O. Parkes for his help in preparing the manuscript. This work was supported by CNRS (Centre National de la Recherche Scientifique), University Paris 7, and short-term fellowships from CNR (Consiglio Nazionale delle Ricerche) and HFSPO (Human Frontier Science Program Organization) to F.G.

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