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Effect of Hydrogen Peroxide on Antioxidant Enzyme Activities in *Saccharomyces cerevisiae* Is Strain-Specific

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Abstract—The effect of hydrogen peroxide on the survival and activity of antioxidant and associated enzymes in *Saccharomyces cerevisiae* has been studied. A difference found in the response of wild-type yeast strains treated with hydrogen peroxide was probably related to the different protective effects of antioxidant enzymes in these strains. Exposure of wild-type YPH250 cells to 0.25 mM H_2O_2 for 30 min increased activities of catalase and superoxide dismutase (SOD) by 3.4- and 2-fold, respectively. However, no activation of catalase in the EG103 strain, as well as of SOD in the YPH98 and EG103 wild strains was detected, which was in parallel to lower survival of these strains under oxidative stress. There is a strong positive correlation ($R^2 = 0.95$) between activities of catalase and SOD in YPH250 cells treated with different concentrations of hydrogen peroxide. It is conceivable that catalase would protect SOD against inactivation caused by oxidative stress and *vice versa*. Finally, yeast cell treatment with hydrogen peroxide can lead to either a H_2O_2-induced increase in activities of antioxidant and associated enzymes or their decrease depending on the H_2O_2 concentration used or the yeast strain specificity.

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Key words: *Saccharomyces cerevisiae*, hydrogen peroxide, catalase, superoxide dismutase

Microorganisms such as yeasts have evolved to survive stressful changes in their environment. Sudden challenge can result in disturbance of cellular functions and even growth arrest [1-3]. Clearly, cells respond rapidly and modify their internal system for prevention of death. Adaptive response usually demands perturbation of genomic expression. Among other stressful conditions, oxidative stress has been well studied in microorganisms [4-8].

In *Escherichia coli* bacteria, most genes induced by oxidative stress are grouped into two regulons, *oxyR* and *soxRS* [5, 6, 8, 9]. In response to hydrogen peroxide exposure, *E. coli* expresses about 30 genes, which include nine genes of the *oxyR* regulon and several genes of the *soxRS* regulon [8, 10]. The yeast *Saccharomyces cerevisiae* possesses two different but overlapping stimulons responsible for cell defense against hydrogen peroxide and superoxide anion [4, 11]. The adaptive response to H_2O_2 in *S. cerevisiae* is under control of Yap1 and Skn7 proteins, and it involves a change in the expression of at least 167 proteins [12]. Cytosolic catalase T, Cu,Zn- and Mn-superoxide dismutases (SOD), glutathione reductase (GR), and glucose-6-phosphate dehydrogenase (G6PDH) are among them [12, 13].

Many previous investigations on adaptive response of microorganisms to oxidative stress are focused on stress-inducible genes and proteins, but not usually on the activities of particular enzymes [2, 8, 10, 12, 13]. Although H_2O_2 effect can be observed by measurement of mRNA or total cell protein levels, the final step in cell adaptive response is the activity of the respective enzymes. In our previous work, we have found that the activity of SOD and G6PDH—members of *soxRS* regulon in *E. coli*—is increased by H_2O_2 [14, 15]. However, no change in transcription level of the *zwf* gene (encoding G6PDH) was found in studies evaluating mRNA levels in *E. coli* cells treated with H_2O_2 [8, 10]. This fact is not the only example of discrepancy existing between results obtained by different researchers [16]. That is why, in order to expand our understanding of cell adaptive response to stress, it is important to use distinct experimental approaches from

Abbreviations: G6PDH) glucose-6-phosphate dehydrogenase; GR) glutathione reductase; ICDH) isocitrate dehydrogenase; SOD) superoxide dismutase.

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detection of gene expression to measurement of activity of protective enzymes.

The aim of the present work was to study the effect of hydrogen peroxide on cell survival and activity of antioxidant and associated enzymes in several *S. cerevisiae* wild-type strains. We also investigated the role of catalase in cell defense against hydrogen peroxide in the exponential phase of growth, using different mutants defective in one or two catalase forms.

MATERIALS AND METHODS

Yeast strains and chemicals. *Saccharomyces cerevisiae* strains used in this study were as follows: YPH250 (*MAT*a *trp1-Δ1 his3-Δ200 lys2-801 leu2-Δ1 ade2-101 ura3-52*), YTT7 (YPH250 *Δctt1::URA3*), YIT2 (YPH250 *Δcta1::TRP1*), and YWT1 (YPH250 *Δcta1::TRP1 Δctt1::URA3*) kindly provided by Dr. Y. Inoue (Kyoto University, Japan); YPH98 (*MAT*a *ura3-52 lys2-801^{amber} ade2-101^{ochre} trp1-Δ1 leu2-Δ1*) and EG103 (*MAT*a *leu2-3,112 his3Δl trp1-289a ura3-52*) kindly provided by Dr. B. Toledano (Biologie Moleculaire Systemique DBJC, France); EG103 (*MATα leu2-3,112 his3Δl trp1-289a ura3-52*) kindly provided by Dr. E. B. Gralla (University of California, Los Angeles, USA).

Glucose-6-phosphate, phenylmethylsulfonyl fluoride (PMSF), isocitrate, and cycloheximide were obtained from Sigma (USA); NADPH, NADP, NAD, oxidized glutathione, *N*,*N*,*N′*,*N′*-tetramethylethylenediamine (TEMED), and quercetin were from Reanal (Hungary). Inorganic chemicals were obtained from Reakhim (Russia). Medium for microorganism cultivation was from Biogene (Great Britain).

Growth conditions and cell extracts. Cells were grown to reach middle exponential phase (A_{600} ~0.4) at 28°C with shaking at 120 rpm in liquid medium containing 1% yeast extract, 2% bactopeptone, and 1% glucose (YPD). For experiments, cells were harvested by centrifugation at 7000*g* for 5 min and resuspended in 50 mM potassium phosphate (K-phosphate) buffer (pH 7.0) to an A_{600} of 0.1. Aliquots of experimental culture were exposed to hydrogen peroxide, followed by incubation of cultures at 28°C for 30 min. To inhibit protein synthesis, cells were pre-exposed to cycloheximide (15 μM) for 60 min.

Cells from the experimental cultures were harvested by the abovementioned method and washed twice with 50 mM K-phosphate buffer (pH 7.0). The yeast pellets were resuspended in lysis buffer (50 mM K-phosphate, pH 7.0, 1 mM PMSF, 0.5 mM EDTA). The cell suspensions were vortexed for 15 cycles of 1 min of vortexing with one volume of glass beads (450-500 μm) (Sigma) followed by 1 min of cooling on ice. Cell debris was removed by centrifugation for 15 min at 15,000*g* and 4°C. The cell extract was kept on ice for immediate use.

Cell survival after hydrogen peroxide exposure was monitored by measuring colony-forming units after 48 h of growth on YPD agar plates at 28°C.

Enzyme activity assays. The activity of superoxide dismutase (SOD) was assayed at 406 nm as the inhibition of quercetin oxidation by superoxide anion [17, 18] in medium containing (final concentrations): 30 mM Tris-HCl buffer (pH 9.0), 0.5 mM EDTA, 0.8 mM TEMED, 50 μM quercetin, and 1-50 μl of cell extract in a final volume of 2.0 ml. One unit of SOD activity was defined as the amount of soluble protein of supernatant that inhibited the maximal rate of quercetin oxidation by 50%. The activity of glucose-6-phosphate dehydrogenase (G6PDH) was measured by monitoring NADP reduction in reaction medium containing 50 mM K-phosphate buffer (pH 7.0), 0.5 mM EDTA, 5.0 mM $MgCl_2$, 0.2 mM NADP, 2.0 mM glucose-6-phosphate, and 30 μl of supernatant in a final volume of 1 ml. The activity of glutathione reductase (GR) was measured by following the consumption of NADPH in reaction medium containing 50 mM K-phosphate buffer (pH 7.0), 0.5 mM EDTA, 1.0 mM oxidized glutathione, 0.25 mM NADPH, and 50 μl of supernatant in a final volume of 1 ml. The activity of isocitrate dehydrogenase (ICDH) was assayed by monitoring NAD reduction in medium containing 50 mM K-phosphate buffer (pH 7.0), 0.5 mM EDTA, 0.25 mM NAD, 0.5 mM isocitric acid, and 100 μl of cell extract in a final volume of 1 ml. NADPH oxidation and NAD or NADP reduction by respective enzymes were registered at 340 nm and the extinction coefficient for these coenzymes of 6.22 $mM^{-1}\cdot cm^{-1}$ was used [17, 18]. Catalase activity was measured by monitoring the disappearance of hydrogen peroxide at 240 nm using the extinction coefficient for hydrogen peroxide of 39.4 $M^{-1}\cdot cm^{-1}$ [17, 18]. The activity of catalase was assayed in 2 ml of medium containing 50 mM K-phosphate buffer (pH 7.0), 0.5 mM EDTA, 10 mM hydrogen peroxide, and 100 μl of cell extract. One unit of G6PDH, GR, ICDH, and catalase activity is defined as the amount of supernatant protein that utilizes or produces 1 μmol of substrate or product per minute. The reactions were started by addition of cell free extract. All activities were measured at 25°C and expressed per milligram of soluble protein in supernatant.

Protein concentration and statistical analysis. Protein concentration was determined by the Coomassie brilliant blue G-250 dye-binding method [19] with bovine serum albumin as the standard. Experimental data are expressed as the mean value of 4-8 independent experiments ± the standard error of the mean (SEM), and statistical testing used Student's *t*-test.

RESULTS

Sensitivity of *S. cerevisiae* to H_2O_2. It is well known that different strains of microorganisms may have various

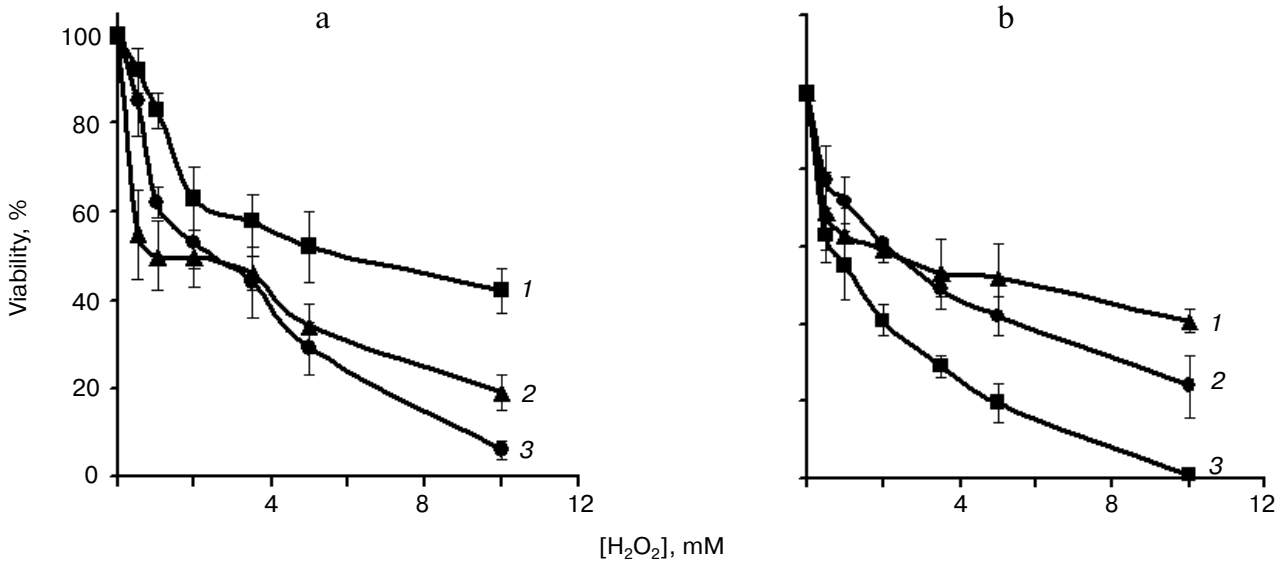

Fig. 1. Viability of exponentially growing *S. cerevisiae* cells under treatment with different H_2O_2 concentrations for 30 min. a) Strains YPH250 (*1*), YPH98 (*2*), and EG103 (*3*); b) strains YIT2 (*1*), YTT7 (*2*), and YWT1 (*3*) (n = 4-6).

sensitivity to oxidants [14, 15, 20, 21]. Therefore, we were interested in determining the effect of hydrogen peroxide on survival of several wild strains of the yeast *S. cerevisiae*. As seen in Fig. 1a, yeast survival decreased with increasing hydrogen peroxide concentration in all cases, but the effect depended on strain. Survival of *S. cerevisiae* YPH250 cells was not affected significantly at a H_2O_2 concentration of 0.5 mM. In the case of EG103 and YPH98, however, the viability of cells treated with 0.5 mM H_2O_2 amounted to 85 and 62% of the untreated cell viability, respectively. There was no significant difference between sensitivities of the different wild-type cells to hydrogen peroxide at concentrations 1.0-3.5 mM. For example, exposure to 3.5 mM H_2O_2 resulted in about 45-55% reduction in the survival of all three strains. At higher concentrations, *S. cerevisiae* YPH250 cells showed a higher resistance to hydrogen peroxide than the other two strains. Cells treated with 10 mM H_2O_2 resulted in a reduction to 42% in the survival of YPH250 strain, whereas EG103 and YPH98 strains demonstrated only 6 and 19% of the cell viability, respectively. It should be noted that EG103 cells showed significantly higher resistance to hydrogen peroxide at low concentrations and lower tolerance at high concentrations compared to YPH98 cells. On average, YPH250 cells survived better than YPH98 and, in turn, YPH98 indicated higher viability than EG103 after exposure to hydrogen peroxide. This may be connected with differences in various antioxidant statuses of these strains.

The susceptibility to hydrogen peroxide of catalase-deficient mutants is shown in Fig. 1b. Yeast strains used in this study were isogenic derivatives from wild type YPH250: YTT7 (*Δctt1* – defective in cytosolic catalase), YIT2 (*Δcta1* – defective in peroxisomal catalase), and catalase-deficient mutant YWT1 (*Δctt1Δcta1*). It has been previously reported that there is no marked difference in the sensitivity of the wild-type and catalase-deficient cells to hydrogen peroxide in early exponential growth phase [22]. Figure 1b shows the various viabilities in each mutant in response to H_2O_2 challenge in the middle of exponential phase of growth. Survival of the strain lacking the *CTT1* gene (Fig. 1b) was similar to that of the wild type YPH250 (Fig. 1a) at H_2O_2 concentrations ranging from 0.5 to 5.0 mM. A significant difference ($p < 0.05$) between the viabilities of YTT7 and YPH250 was seen only at the highest concentration used, 10 mM H_2O_2. In contrast, YIT2 (Fig. 1b) showed 1.3-fold lower sensitivity to hydrogen peroxide at concentrations 0.5-1.0 mM compared to the parent strain YPH250 (Fig. 1a). At higher concentrations, wild-type and *CTA1*-deficient cells showed virtually the same sensitivity to H_2O_2. Survival of the catalase-deficient strain was significantly lower than that of the wild-type and single-mutant catalase-deficient cells at all H_2O_2 concentrations used. It should be noted that very few of the double-mutant cells were able to survive after treatment with 10 mM H_2O_2.

Activity of antioxidant enzymes. A high level of *de novo* synthesis of catalase T, both SODs, and other proteins in *S. cerevisiae* as a result of hydrogen peroxide exposure was previously reported [12, 13]. However, it is documented that in some cases protein synthesis does not correlate with expression of respective genes and enzyme activities [14-16]. We next examined the activity of antioxidant enzymes in the wild-type and catalase-deficient cells under hydrogen peroxide stress. The data presented in Fig. 2 show that exposure of wild-type YPH250 cells to H_2O_2 for 30 min changed catalase activity in a

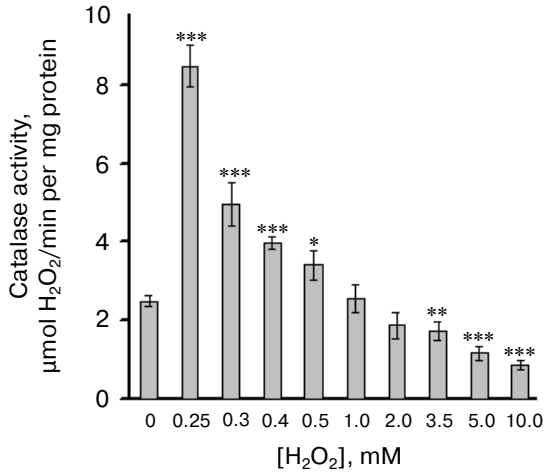

Fig. 2. Catalase activity in *S. cerevisiae* YPH250 cells treated with different H_2O_2 concentrations for 30 min. * Significantly different from respective values for the control cells (without H_2O_2) with $p < 0.05$, $^{**}p < 0.025$, and $^{***}p < 0.001$ ($n = 4$-6).

dose-dependent manner. The activity reached a maximum value (3.4-fold higher than control) during incubation with as little as 0.25 mM H_2O_2 and remained significantly higher than control at 0.3-0.5 mM H_2O_2. There was no marked difference between cells untreated and incubated with 0.5-2.0 mM H_2O_2, but at higher concentrations, the activity was reduced to 69% (3.5 mM), 48% (5.0 mM), and 35% (10 mM) in comparison with the control. It should be noted that catalase activity increased by 2-fold in YPH98 in response to cell treatment with 0.25 mM H_2O_2, but did not change in EG103 at any concentrations used (not shown).

In our previous studies, it was shown that there is a strong relationship between catalase and SOD activities under different experimental conditions [17, 18]. In this experiment, SOD activity in YPH250 (Fig. 3) depended on the dose of hydrogen peroxide in a similar to catalase manner (Fig. 2). Data presented in Fig. 3 show that the highest activity of SOD (2-fold higher than control) in the wild-type cells was observed at 0.25 mM H_2O_2, but at higher concentrations the activity was reduced, being 38% of control in the presence of 5 and 10 mM H_2O_2. At the same time, no SOD activation was found in the two other wild strains YPH98 and EG103 under any used conditions (not shown). The effect of low doses of hydrogen peroxide on catalase and SOD in YPH250 cells was cancelled by cycloheximide, an inhibitor of protein synthesis in eukaryotes (not shown).

Figure 3 also demonstrates the effect of hydrogen peroxide on SOD activity in catalase-deficient YWT1 cells. In this case, H_2O_2 induced a dose-dependent decrease in SOD activity to 34% at the highest concentration of 10 mM. The activity of SOD was 1.5-fold lower ($p < 0.001$) in control catalase-deficient cells, compared to the wild type. However, the activity in catalase-deficient (YWT1) and wild-type cells incubated with 3.5-10 mM H_2O_2 was virtually the same. Because there is no induction of SOD activity by hydrogen peroxide in catalase-deficient strain, one may suppose that in some way catalase is involved in the observed effect of SOD activation in the parent YPH250 strain.

Saccharomyces cerevisiae posses two catalases, peroxisomal catalase A and cytosolic catalase T [7, 22, 23]. To determine which form of the enzyme could be associated with the increment of SOD activity in the wild type, the single-mutant catalase-deficient strains were used in further experiments. Table 1 indicates catalase and SOD activity in the two mutants under oxidative stress induced by 0.5 mM H_2O_2 for 30 min. Catalase activity in control cells of the strain lacking the *CTT1* gene was 2.5-fold lower than that in the strain defective in the *CTA1* gene. However, because hydrogen peroxide increased catalase activity by 281% in *Δctt1* cells and only by 120% in *Δcta1* cells, there is no significant difference between catalase activities in both single mutants after H_2O_2 treatment. This is in agreement with the previous study, which reported that transcription of both *CTT1* and *CTA1* genes was induced by hydrogen peroxide [24].

Data presented in Table 1 demonstrates that SOD activity is similar in control cells of the two catalase-deficient strains. Under hydrogen peroxide exposure *Δctt1* and *Δcta1* mutants also showed similar patterns of SOD activity, increasing by 1.2- and 1.3-fold, respectively. It may be supposed that both catalases play a similarly important role in SOD activation of YPH250 towards stress induced by hydrogen peroxide.

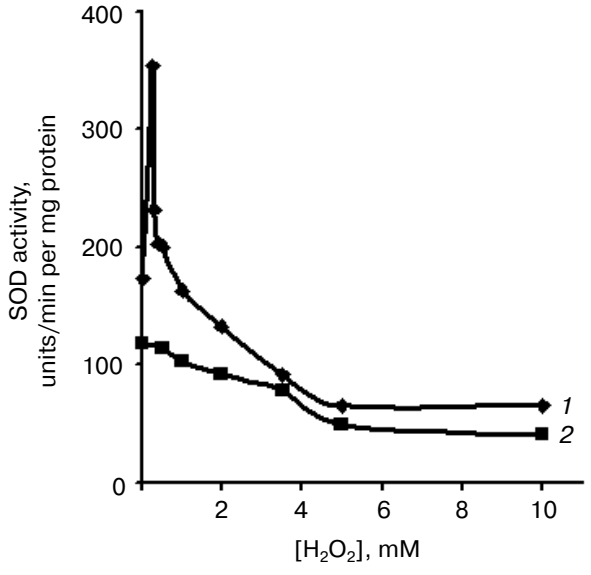

Fig. 3. SOD activity in *S. cerevisiae* YPH250 (*1*) and YWT1 (*2*) cells treated with different H_2O_2 concentrations for 30 min (n = 4-8).

Table 1. Activities of catalase and superoxide dismutase in *S. cerevisiae* strains YIT2 (*ΔCTA1*) and YTT7 (*ΔCTT1*) under cell treatment with 0.5 mM H_2O_2 for 30 min

Enzyme	YIT2		YTT7	
	control	stress	control	stress
Catalase, μmol H_2O_2/min per mg protein	3.00 ± 0.22	3.61 ± 0.18*	1.21 ± 0.04	3.40 ± 0.17***
SOD, units/mg protein	193 ± 7	229 ± 10*	203 ± 7	282 ± 19**

Note: * Significantly different from respective values for the control cells (without H_2O_2) with $p < 0.025$, ** $p < 0.005$, and *** $p < 0.001$ (n = 4-7).

Table 2. Activity of glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G6PDH), and isocitrate dehydrogenase (ICDH) (nmol NAD(P)H/min per mg protein) in *S. cerevisiae* strains YPH250 (wild type) and YWT1 (*ΔCTA1ΔCTT1*) under cell treatment with 0.5 mM H_2O_2 for 30 min

$[H_2O_2]$, mM	YPH250		YWT1		
	GR	G6PDH	GR	G6PDH	ICDH
0	35.4 ± 2.3	47.2 ± 1.0	40.6 ± 1.0	55.6 ± 1.2	5.60 ± 0.23
0.5	36.2 ± 1.7	41.8 ± 1.8**	41.6 ± 0.4	50.0 ± 3.7	4.72 ± 0.31***
1.0	35.6 ± 2.0	40.0 ± 1.8***	42.0 ± 0.4	48.0 ± 3.0**	4.58 ± 0.32**
2.0	31.8 ± 0.9	33.0 ± 0.7****	43.1 ± 1.5	47.5 ± 4.3*	4.26 ± 0.3***
3.5	33.0 ± 2.3	34.0 ± 1.2****	42.0 ± 1.1	47.3 ± 3.1**	3.82 ± 0.41***
5.0	31.4 ± 0.5	31.2 ± 1.2****	39.4 ± 1.5	43.0 ± 2.2****	3.65 ± 0.14****
10.0	25.4 ± 1.7***	25.0 ± 2.0****	11.7 ± 0.6****	30.7 ± 0.9****	2.05 ± 0.06****

Note: * Significantly different from respective values for the control cells (without H_2O_2) with $p < 0.05$, ** $p < 0.025$, *** $p < 0.005$, and **** $p < 0.001$ (n = 4-8).

Activity of enzymes associated with antioxidant. It is well known that the enzymes GR, G6PDH, and ICDH are functionally associated with antioxidant ones and are essential for adaptation to oxidative stress [5, 6, 12, 13, 17, 18, 25]. On the other hand, it has been shown that some of these enzymes can be inactivated by oxidants [26-28]. Table 2 shows the results of activity measurement of GR, G6PDH, and ICDH in the wild-type and catalase-deficient cells exposed to different H_2O_2 concentrations for 30 min. The activity of GR and G6PDH in untreated cells of the wild type is slightly, but significantly lower (15 and 18%, respectively) than those in catalase-deficient cells. Surprisingly, in contrast to double mutant, ICDH activity was not detected in the parent strain at all. In both strains, treatment with 0.5-5.0 mM H_2O_2 did not change GR activity. However, yeast exposure to 10 mM H_2O_2 caused a sharp loss in GR activity to 72 and 29% in the wild-type and catalase-deficient strain, respectively. The activity of G6PDH decreased significantly in response to hydrogen peroxide exposure at all H_2O_2 concentrations used. It can be seen that G6PDH activity of both strains was reduced approximately 2-fold by treatment with the highest concentration used, 10 mM H_2O_2. As mentioned above, in contrast to catalase-deficient cells, we did not find ICDH activity in the parent strain under any conditions used. The effect of hydrogen peroxide on ICDH activity in double mutant was similar to that observed for G6PDH inhibition.

DISCUSSION

Oxidative stress seems to be one of the most common stresses experienced by microorganisms in their environment. In this case, antioxidant mechanisms act to protect cells against oxidation. Earlier we observed different patterns of sensitivity to oxygen in bacterial strains that are due to their various antioxidant capacities [14, 21]. The results obtained in this study show that three *S. cerevisiae* wild strains are different in their tolerance to hydrogen peroxide (Fig. 1a). The higher survival of YPH250 under hydrogen peroxide exposure may be explained by the

compensatory elevation of catalase (Fig. 2) and SOD (Fig. 3) activities in the cells treated with H_2O_2. It should be noted that we did not observe any catalase activation in EG103 and SOD in YPH98 and EG103 wild strains demonstrating lower availability under exposure to hydrogen peroxide.

Japanese researchers suggested that in early exponential growth phase, catalase may not act to defend *S. cerevisiae* cells against hydrogen peroxide [22]. However, we observed higher sensitivity to hydrogen peroxide in catalase-deficient cells harvested in the middle exponential phase than that in wild type (Fig. 1b). This indicates that catalase plays an important role defending yeast against hydrogen peroxide under conditions used in this study. The data presented in Fig. 4 confirms the above-mentioned suggestion. The figure demonstrates a strong positive correlation between catalase activity in the untreated control cells of all yeast strains investigated and their survival after exposure to 10 mM H_2O_2. In addition, the low level of cell survival in catalase-deficient strain (YWT1) demonstrated strong positive correlation with decreased SOD activity under hydrogen peroxide stress (Fig. 5).

The above observations suggest that both catalase and SOD play an important role in yeast survival under oxidative stress induced by hydrogen peroxide. This suggestion is confirmed by studies of characterized proteins whose synthesis is elevated in *S. cerevisiae* upon cells treatment with low doses of hydrogen peroxide [12, 13].

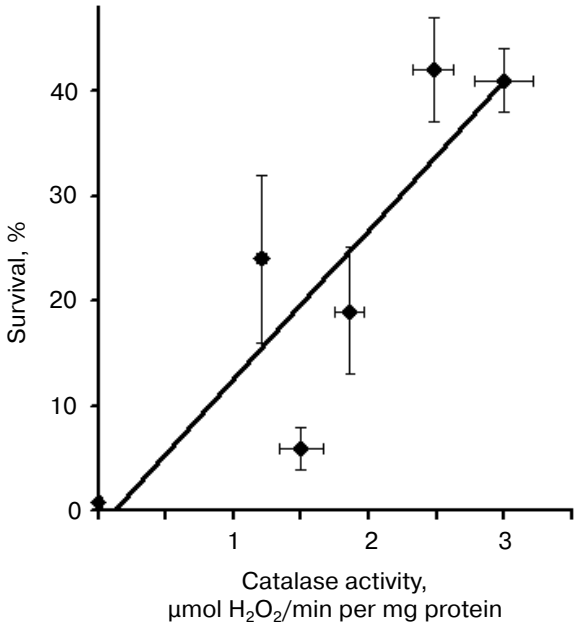

Fig. 4. Correlation analysis of data obtained with all *S. cerevisiae* strains investigated: correlating between catalase activity in untreated control cells (without H_2O_2) and survival of cells exposed to 10 mM H_2O_2 for 30 min (n = 4-6, R^2 = 0.75).

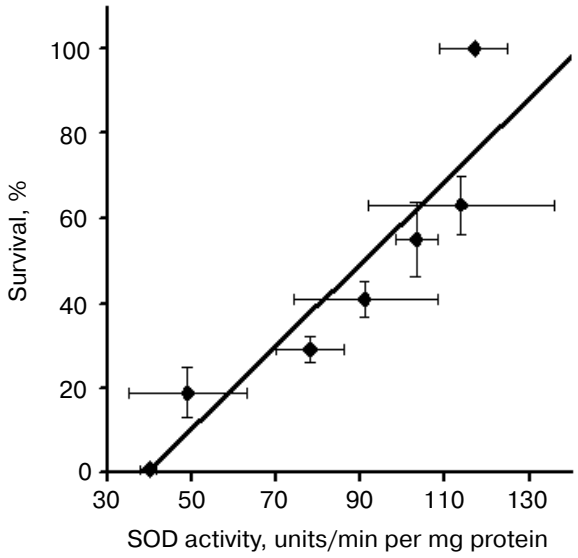

Fig. 5. Correlation analysis of data obtained with *S. cerevisiae* YWT1 cells treated with different H_2O_2 concentrations for 30 min: correlating between SOD activity and cell survival (n = 4-8, R^2 = 0.84).

Although the authors observed strong elevation in *de novo* protein synthesis of catalase T, Cu,Zn-SOD, and Mn-SOD by 14.7-, 4.3-, and 5.9-fold, respectively, in YPH98, we found that hydrogen peroxide caused only a 2-fold increase in catalase activity but did not change SOD activity in the same strain under the same conditions. It should be noted that French researchers also found no changes in the level of catalase A [12, 13], but in our experiments, catalase activity in the strain lacking the *CTT1* gene was significantly increased by hydrogen peroxide (Table 1). As for induction of catalase A activity in the respective catalase-deficient single mutant, results obtained in the present study confirmed the previous investigation, which reported that transcription of both *CTT1* and *CTA1* genes was induced by hydrogen peroxide [24]. Although reorganization of genomic expression and protein synthesis is one of the most important aspects of cellular adaptation to oxidative stress [2], the respective activity of certain enzymes is the final step of response to any environmental changes. It is well known that not only transcription and translation, but other mechanisms may be involved in enzyme activity regulation. That is why measurement of mRNA or total cell protein levels can describe only one among other important stages of cellular adaptive response.

One more interesting fact can be observed in YPH250 cell response to oxidative stress induced by different concentrations of hydrogen peroxide. As seen in Fig. 6, there is a strong positive correlation between catalase and SOD activities. A similar relationship between the two enzymes was found in our previous *in vivo* studies

[17, 18]. The above observations may have some physiological meaning. First of all, SOD produces hydrogen peroxide which serves as a substrate for catalase. In addition, it is well known that both enzymes prevent inactivation by hydrogen peroxide of SOD and by superoxide of catalase, defending each other against oxidation in the active centers [9, 29]. On the other hand, H_2O_2 is known to be causative of Cu,Zn-SOD carbonylation and, in turn, of Cu,Zn-SOD inactivation [30]. If it is taken into account that Cu,Zn-SOD represents up to 90% of the total SOD activity in the yeast cell [31], the decrease in SOD activity after treatment with high doses of hydrogen peroxide (Fig. 3) should be associated with Cu,Zn-SOD inactivation.

Lower catalase activity in cells exposed to 3.5-10 mM H_2O_2 (Fig. 2) could be explained by inactivation of catalase *in vivo* by high concentrations of substrate. Earlier *in vivo* and *in vitro* experiments demonstrated that in *E. coli* catalase activity did not depend linearly on hydrogen peroxide concentration [32]. The results were interpreted from the point of view of enzyme inactivation by high peroxide concentrations. In addition, it has been shown that exponentially growing *E. coli* strain lacking catalase HPI had significantly higher intracellular hydrogen peroxide levels compared to the parent strain [20]. Therefore, lower SOD activity in catalase-deficient *S. cerevisiae* cells compared with the wild strain may be associated with elevated intracellular H_2O_2 levels.

It is very attractive to suppose that catalase and SOD are somehow involved in regulation of each other. This suggestion may partially follow from Fig. 3, which clearly shows the absence of SOD activity increase in catalase-deficient cells treated with 0.25-0.5 mM H_2O_2 in contrast to that in the wild-type strain. Similar increase in SOD activity of the two catalase-deficient single mutants (Table 1) let us also conclude that both catalase isozymes may play some role in activation of SOD by low doses of hydrogen peroxide. In addition, using cycloheximide to inhibit protein synthesis cancelled the induction of catalase and SOD by low doses of hydrogen peroxide in YPH250. This is in agreement with data from other laboratories demonstrating that yeast response to peroxide stress requires synthesis of protective enzymes *de novo* [11, 33].

On the basis of the previous report [20] and our observations, one may suppose that some maximum intracellular H_2O_2 concentration might exist, and exceeding that level may lead to SOD and even catalase inactivation. On the other hand, H_2O_2 at concentrations ranging between steady-state intracellular level and this virtual maximum value causes adaptive activation of catalase and SOD that may reflect a complicated activation/inactivation balance in the cell which, in turn, strongly depends on intracellular levels of H_2O_2 and/or products of its metabolism. It is suggested that these critical concentrations of the oxidant, which can be "sensed" by cells, depend on many circumstances; for example, growth phase, conditions of yeast cultivation, and strain specificity.

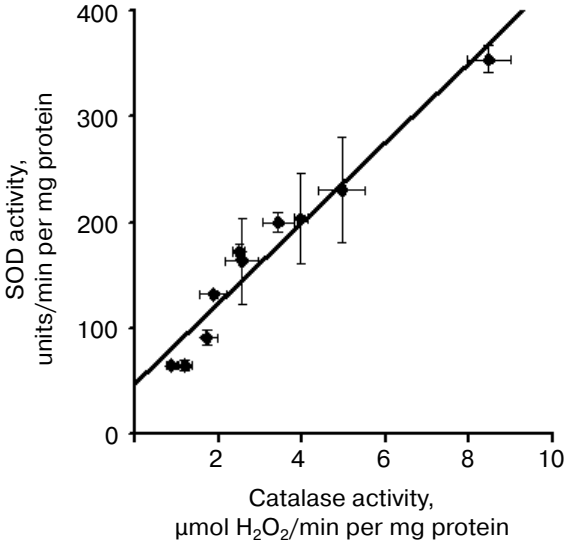

Fig. 6. Correlation analysis of data obtained with *S. cerevisiae* YPH250 cells treated with different H_2O_2 concentrations for 30 min: correlating between SOD and catalase activities (n = 4-6, $R^2 = 0.95$).

The enzymes known as associated with antioxidant ones contribute to oxidative stress protection, providing necessary intracellular oxidant/antioxidant balance in the cell [5, 6, 12, 13, 17, 18, 25]. Our study shows that the activity of G6PDH and GR is significantly higher in catalase-deficient yeast than that in the parent strain. In addition, we detected no ICDH activity in the wild type in contrast to its derivative lacking catalases. It is likely that these enzymes help to correct the imbalance in the redox state and might compensate partially for the lack of catalase and diminished SOD activity of the defective strain. However, cell treatment with hydrogen peroxide caused decrease in the activity of GR, G6PDH, and ICDH with increasing concentration of hydrogen peroxide, demonstrating the patterns of linear inhibition for G6PDH and ICDH. Data obtained in the present experiment are not in line with studies [22, 25], which reported that sublethal concentrations of H_2O_2 induced GR and G6PDH. This discrepancy may be explained by the distinct conditions used by different laboratories for oxidative stress induction. It is possible that under conditions used in this study the intracellular oxidant/antioxidant balance is more shifted to the oxidant direction causing decrease in the activity of GR, G6PDH, and ICDH. Although enzymes associated with antioxidant are known as a major cellular source of reducing power, some of them are susceptible to oxidation [27, 28]. It is well known that hydrogen peroxide can inactivate ICDH via oxidation of sulfhydryl groups, which are known to be responsible for the catalytic function of the enzyme [26].

In our previous study, decreased G6PDH activity correlated with increase of carbonyl protein levels in wild and catalase-deficient yeast strains, and a strong positive correlation between catalase and G6PDH activities was observed [27]. In this experiment, we have found positive correlations between catalase and G6PDH (R^2 = 0.71) and catalase and GR (R^2 = 0.77) activities in parent YPH250 strain and positive correlations between SOD and G6PDH (R^2 = 0.78) and SOD and ICDH (R^2 = 0.84) activities in catalase-deficient YWT1 cells treated with different H_2O_2 concentrations (not shown). Hence, it can be supposed that hydrogen peroxide or products of its metabolism caused oxidative damage to GR, G6PDH, and ICDH under stress conditions used in this study and catalase and SOD play an important role in protection of these enzymes from inactivation.

In summary, our data show that *S. cerevisiae* strains YPH250, YPH98, and EG103 demonstrate different sensitivity towards hydrogen peroxide, which may be connected with their various antioxidant potentials. The results obtained in this study also show that there is a strong positive correlation between catalase and SOD activities in *S. cerevisiae* YPH250 cells treated with different concentrations of hydrogen peroxide. Hydrogen peroxide can cause an increase as well as a decrease in antioxidant and associated enzymes, depending on its concentration and the yeast strain peculiarities.

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