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EXPERIMENTAL ARTICLES

Heat Shock–Induced Changes in the Respiration of the Yeast *Saccharomyces cerevisiae*

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Abstract—The incubation of *Saccharomyces cerevisiae* at elevated temperature (45°C) stimulated the respiration of yeast cells and decreased their survival rate. The respiration-deficient mutant of this yeast was found to be more tolerant to the elevated temperature than the wild-type strain. At the same time, the cultivation of the wild-type strain in an ethanol-containing medium enhanced the respiration, catalase activity, and thermotolerance of yeast cells, as compared with their growth in a glucose-containing medium. It is suggested that the enhanced respiration of yeast cells at 45°C leads to an intense accumulation of reactive oxygen species, which may be one of the reasons for the heat shock–induced cell death.

Key words: *Saccharomyces cerevisiae*, thermotolerance, respiration- deficient mutant, reactive oxygen species.

Heat shock induces global changes in the cell metabolism by impairing the cell membranes and stimulating the denaturation and aggregation of cell proteins. Heat-shock proteins (HSPs) and trehalose, synthesized in response to heat shock [1, 2], are involved in the cell protection from heat-induced damage.

There is increasing evidence that heat shock induces the generation of active oxygen species (AOS) in *Saccharomyces cerevisiae* cells [3], such as superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^\bullet$). The accumulated AOS impair cell membranes, proteins, and DNA, eventually causing cell death [4].

The reason for the generation of AOS in response to heat shock remains unclear, although it is known that, at a normal temperature, cell respiration is the main source of AOS [5]. The yeast *S. cerevisiae* is a facultative aerobe, whose growth is due to both cell respiration and fermentation. Mutants deficient in respiration (rho$^-$ mutants) can be easily derived from this yeast.

The aim of the present work was to study the role of respiration in the yeast tolerance to heat shock at 45°C.

MATERIALS AND METHODS

Experiments were performed with *Saccharomyces cerevisiae* α w$^+$303-1B (the wild-type strain), which was kindly provided by F. Lacroute from the Center of Molecular Genetics in Gif-sur-Yvette (France), and the respiration- deficient mutant rho$^-$ α w$^-$303-1B/S, which was derived by incubating the strain w$^+$303-1B at 45°C for 30 min. Under such incubation conditions, about 50% of the surviving cells were rho$^-$ mutants. The rho$^-$ phenotype mutants were identified by using conventional methods [6].

Material for inoculation was grown at 30°C for two days on solid YEPD medium containing (g/l) yeast extract, 5; peptone, 29; glucose, 20; and agar, 15. Yeast cells were inoculated either into liquid YEPD medium or into YEPE medium, which contained 20 ml/l ethanol instead of glucose, and cultivated for 16 h on a temperature-controlled shaker. Then an aliquot of this overnight culture was inoculated into the fresh nutrient medium and incubated to a culture density of 2×10^7 cells/ml.

To estimate the respiration rate of yeast cells grown in YEPD or YEPE medium, they were harvested by centrifugation at 5000 *g* for 5 min, resuspended in 1 ml of fresh YEPD medium, and cooled at 4°C for 15 min. Aliquots of this suspension 50 to 20 µl in volume were introduced into the temperature- controlled measuring cell of a Clark-type oxygen electrode containing 1.4 ml K,Na phosphate buffer (pH 7.0) with 50 mM of glucose. The respiration of yeast cells was measured at 30, 45, and 50°C for 10 min and expressed in nmoles of oxygen consumed per min per 10^7 cells, taking into account the solubility of oxygen in water at different temperatures [7].

In survival experiments, 1-ml aliquots of the above yeast suspension were transferred to test tubes placed in a temperature-controlled shaker (110 rpm) and exposed to 45°C for 0, 15, 30, and 60 min. After the treatment, the cell suspensions were cooled on ice and plated onto minimal nutrient medium containing (g/l) glucose, 20;

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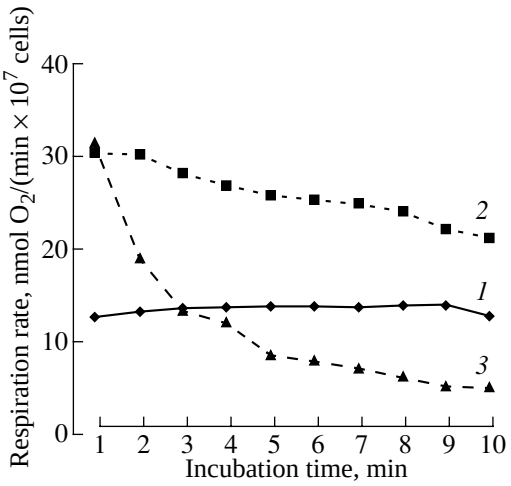

Fig. 1. Dynamics of the respiration of the YEPD-grown *S. cerevisiae* w^+303-1B cells at (*1*) 30, (*2*) 45, and (*3*) 50°C.

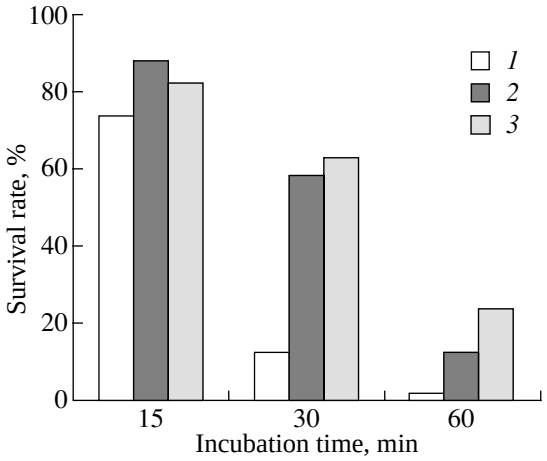

Fig. 2. Effect of heat shock (45°C) on the survival of (*1*) strain w^+303-1B in YEPD medium; (*2*) strain rho^- w^- 303-1B/S in YEPD medium; and (*3*) strain w^+303-1B in YEPE medium.

KH_2PO_4, 0.9; K_2HPO_4, 0.1; $MgSO_4$, 1.0; $(NH_4)_2SO_4$, 1.0; agar, 15.0; and 200 μg/l thiamine. Colonies were enumerated after two days of growth at 30°C. Cell survival was defined as a percentage of the colonies grown after the respective exposure to 45°C with respect to the control.

To assay catalase activity, yeast cells were grown in YEPD or YEPE medium at 30°C to a concentration of 4×10^7 cells/ml, harvested by centrifugation at 5000 *g* for 5 min, washed thrice with K,Na phosphate buffer (pH 7.0), and stored at –20°C for one day to be then used for the preparation of cell homogenate. To disrupt the cell wall, the yeast biomass was resuspended in K,Na phosphate buffer (pH 7.0), frozen in liquid nitrogen, and then ground with quartz sand. Soluble proteins were separated from cell debris by centrifugation at 15000 *g* for 15 min. Catalase activity was assayed polarographically by measuring the evolution rate of oxygen resulting from the decomposition of H_2O_2 by catalase [8]. Measurements were performed at room temperature using the aforementioned Clark-type oxygen electrode. For this, 50 μl of the cell homogenate was added to phosphate buffer in the polarographic cell, and then 10 μ H_2O_2 was added to give a final concentration of 0.0003%. Catalase activity was expressed in nmoles of oxygen liberated from hydrogen peroxide per min per mg protein. Protein was quantified by the method of Lowry *et al.* [9].

All the experiments were performed at least in four replicates.

RESULTS AND DISCUSSION

The rate of oxygen uptake by the wild-type *S. cerevisiae* cells incubated at 30°C changed insignificantly throughout the incubation period (Fig. 1, curve *1*). At an elevated temperature (45°C), the initial rate of oxygen uptake was more than twofold higher than at 30°C (Fig. 1, curve *2*). Then the cell respiration tended to diminish, albeit insignificantly, so that, after 10-min of incubation at 45°C, the respiration rate was well in excess of the control level. Raising the temperature to 50°C increased the rate of cell respiration; however, as early as 3 min after the onset of the experiment, the respiration fell to the control level and continued to gradually decrease in the course of the further incubation (Fig. 1, curve *3*).

The increase in the cell respiration in response to heat shock was also observed by other authors for different yeast organisms [10–12]. It was shown that, during heat shock, the cell loses intracellular ATP and tries to compensate for the loss by enhancing its respiration [13]. On the other hand, the increased respiration must stimulate the generation of AOS, since the AOS production in cells is proportional to the intensity of their respiration [14]. Experiments with the oxidant-sensitive 2',7'-dichlorofluorescein probe revealed a two- to threefold increase in the fluorescence of this probe in the cells exposed to heat shock, indicating a rise in the intracellular level of AOS.

The survival experiments showed that the exposure of *S. cerevisiae* cells to 45°C exerted a strong lethal effect on the cells (Fig. 2). Assuming that the enhanced respiration of heat-treated cells is one of the possible reasons for the generation of AOS, we compared the thermotolerance of the rho^-mutant w^-303-1B/S strain and the wild-type strain w^+303-1B. The wild-type strain (Fig. 2, bar *1*) proved to be much less tolerant to 45°C than the rho^- mutant (Fig. 2, bar *2*). However, similar investigations performed by Gauze and Kuzovkova [15] showed that, at a temperature of 50°C, the survival rate of the rho^- mutants was much lower than that of the wild-type strain. This observation was confirmed in our preliminary experiments on the cell

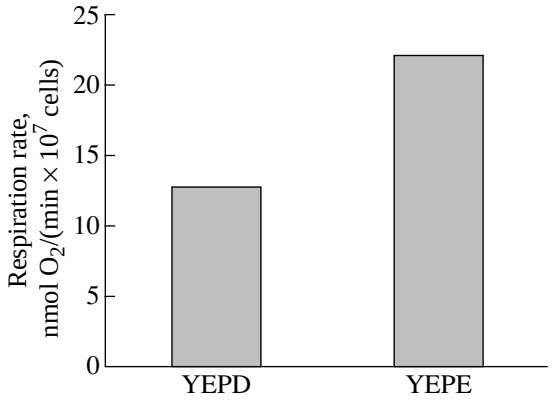

Fig. 3. Respiration of *S. cerevisiae* w^+303-1B cells in YEPD and YEPE media at 30°C.

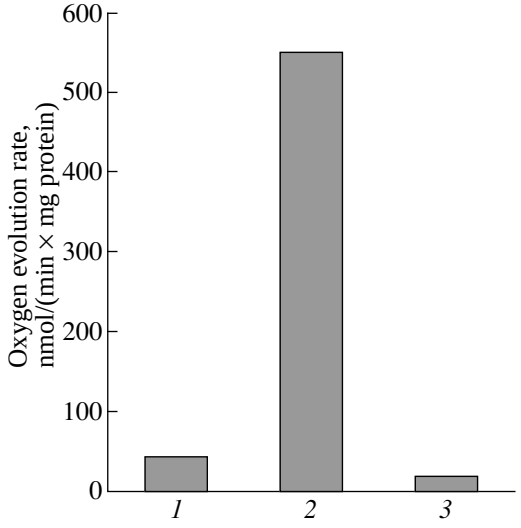

Fig. 4. The catalase activity (30°C) of (*1*) strain w^+303-1B grown in YEPD medium; (*2*) strain w^+303-1B grown in YEPE medium; and (*3*) strain rho^- w^-303-1B/S grown in YEPD medium.

survival at 50°C. Hence, the mechanisms responsible for the damage of cells exposed to 45 and 50°C may substantially differ.

It was reasonable to suggest that one of the possible reasons for the cell death at 45°C is the high intracellular level of AOS due to the enhanced cell respiration (Fig. 1, curve *2*). At 45°C, the rho^- mutant is more thermotolerant than the wild-type strain (Fig. 2, bars *1* and *2*). At 50°C, the initial respiration rate of the wild-type strain was also higher than at 30°C, but it rapidly decreased to below the control level (Fig. 1, curve *3*). Therefore, it can be suggested that, at 50°C, the AOS are not generated so intensively as at 45°C, and cell death may be caused by other factors, such as the direct thermal damage of labile cellular structures. In this case, the rho^- mutant may appear to be more susceptible to thermal damage than the wild-type strain.

In the next set of experiments, we studied the response to heat shock of the wild-type strain grown in YEPE medium containing ethanol (nonfermentable carbon source) instead of glucose. The rate of oxygen consumption by the yeast cells grown in YEPE medium at 30°C was nearly twice that of the cells grown in the medium with glucose (Fig. 3). This phenomenon is known as catabolic repression, because the glucose-growing facultative aerobe *S. cerevisiae* derives energy primarily from glycolysis, which leads to the suppression of respiration [16]. In spite of its more intense respiration in YEPE medium at 30°C, the thermotolerance of the wild-type strain was considerably higher in this medium than in YEPD medium (Fig. 2, bars *1* and *3*).

The increased thermotolerance of *S. cerevisiae* during its growth on nonfermentable carbon sources was also reported by other authors [17–19]. The *S. cerevisiae* cells grown on acetate were more thermotolerant than those grown on glucose. Sanchez *et al.* [17] explained this observation by a higher level of the constitutive synthesis of the heat-shock protein 104. However, Gross and Watson, who comparatively studied thermotolerant and thermosensitive strains, did not observe strong correlation between the enhanced thermotolerance of cells grown on acetate and the level of the synthesis of heat-shock proteins [18, 19].

Inasmuch as heat shock is accompanied by oxidative stress, the antioxidant enzymes superoxide dismutase, catalase, and peroxidase must be essential for the thermotolerance of *S. cerevisiae* cells. Indeed, the expression of the *CTT1* gene, encoding the cytosolic catalase T of *S. cerevisiae*, substantially increased with the temperature rise from 23 to 37°C [20]. Mutants deficient in the synthesis of catalase, peroxidase, and superoxide dismutase were found to be more thermosensitive than the wild-type strains, while the overexpression of the catalase and superoxide dismutase genes leads to thermoresistance [3].

To explain the enhanced tolerance of the respiration-deficient yeast cells in YEPD medium and of the actively respiring yeast cells in YEPE medium to 45°C, we measured the catalase activity of yeast cells. The catalase activity of the wild-type w^+303-1B cells grown in YEPD medium (Fig. 4, bar *1*) was found to be several times lower than during their growth in YEPE medium (Fig. 4, bar *2*). Therefore, the enhanced thermotolerance of the yeast cells grown on a nonfermentable carbon source may be due to the elevated level of antioxidant enzymes, catalase in particular.

To conclude, the data presented in this paper show that the exposure of yeast cells to 45°C stimulates their respiration, which probably leads to the enhanced generation of AOS and eventually kills the cells. The suppressed respiration of the rho^- mutant allows it to tolerate the elevated temperature (45°C) better. On the other hand, the wild-type strain grown at 30°C in the medium with ethanol has a higher respiration and, consequently, a higher level of antioxidant activity than in the medium

with glucose. This makes yeast cells more tolerant to oxidative stress, which accompanies heat shock.

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