Microbiology, Vol. 72, No. 5, 2003, pp. 548–552. Translated from Mikrobiologiya, Vol. 72, No. 5, 2003, pp. 616–620.

EXPERIMENTAL ARTICLES

The Effect of Sodium Malonate on Yeast Thermotolerance

E. G. Rikhvanov[1], N. N. Varakina, T. M. Rusaleva, E. I. Rachenko, and V. K. Voinikov

Siberian Institute of Plant Physiology and Biochemistry, Siberian Division, Russian Academy of Sciences, ul. Lermontova 132, Irkutsk, 664033 Russia

Received April 30, 2002

Abstract—The study of the effect of malonate (an inhibitor of the succinate dehydrogenase complex of the respiratory chain of mitochondria) on the thermotolerance of the fermentative *Saccharomyces cerevisiae* and nonfermentative *Rhodotorula rubra* yeasts showed that malonate augmented the damaging effect of heat shock on the yeasts utilizing glucose (or other sugars) by means of oxidative phosphorylation. At the same time, malonate did not influence, and sometimes even improved, the thermotolerance of the yeasts utilizing glucose through fermentation. The suggestion is made that cell tolerance to heat shock depends on the normal functioning of mitochondria. On the other hand, their increased activity at elevated temperatures may accelerate the formation of cytotoxic reactive oxygen species and, hence, is not beneficial to cells.

Key words: sodium malonate, thermotolerance, mitochondria, yeasts.

Depending on the species, the availability of oxygen, and the concentration of glucose in the medium, yeasts can utilize this sugar either through fermentation or through oxidative phosphorylation. In the presence of oxygen, *Saccharomyces cerevisiae* and related yeasts utilize glucose largely through its fermentation to ethanol. In this case, cell respiration is suppressed (the so-called glucose repression of respiration or the Crabtree effect) [1]. Glucose, even at high concentrations, does not completely suppress cell respiration. Unlike glucose, galactose (and some other sugars) inhibits fermentative processes and enhances the respiration of fermentative yeasts [2]. Other yeasts, such as *Debaryomyces vanriji* (presently *D. vanrijiae*) [3] and *Rhodotorula rubra* [4], utilize glucose and other sugars solely through oxidative phosphorylation.

In our earlier studies, we showed that the inhibition of mitochondria with sodium azide and cyanide (inhibitors of the cytochrome oxidase complex of the mitochondrial respiratory chain) considerably diminished the thermotolerance of the *D. vanrijiae* and *R. rubra* yeasts incubated in the presence of glucose. Under the same conditions, cyanide did not influence the thermotolerance of *S. cerevisiae* [5], whereas azide even enhanced the thermotolerance of the yeasts *S. cerevisiae* and *Candida albicans* [5, 6]. Taking into account the fact that, unlike *S. cerevisiae* and *C. albicans*, the yeasts *D. vanrijiae* and *R. rubra* are unable to ferment glucose, we inferred that the effect of respiratory inhibitors on the thermotolerance of yeasts depends on the way (either oxidative or fermentative) by which these yeasts utilize the source of carbon and energy. Moreover, we suggested that the thermotolerance of actively respiring yeast cells is determined by the activity of mitochondria, so that their impairment is catastrophic to cells [5].

It is known that heat shock enhances the generation of reactive oxygen species (ROS) in cells [7, 8]. Since both azide and cyanide inhibit the antioxidant enzymes catalase and superoxide dismutase (antioxidant enzymes prevent the excessive accumulation of ROS in cells to toxic levels), there is the possibility that azide and cyanide diminish the thermotolerance of *D. vanrijiae* and *R. rubra* through the suppression of the antioxidant enzymes [5].

This work was undertaken to study the effect of sodium malonate on the thermotolerance of the yeasts *R. rubra* and *S. cerevisiae* differing in the way of glucose utilization. The choice of sodium malonate was dictated by the fact that it is a specific inhibitor of the succinate dehydrogenase complex of the respiratory chain of mitochondria [9] and, to the best of our knowledge, does not inhibit antioxidant enzymes.

MATERIALS AND METHODS

Experiments were carried out with two yeasts, *Rhodotorula rubra* Dz40-1, isolated from a hot spring in Buryatia, and *Saccharomyces cerevisiae* Ψ-74-D694, kindly provided by S. Lindquist from the University of Chicago, United States. The yeasts were grown in a YEPD medium containing (g/l) yeast extract, 5; peptone, 10; and glucose, 20. When required, the medium was solidified with 15 g/l agar. During experiments, the yeasts were maintained on a YEPD medium at 30°C.

Cells for inoculation were grown on a shaker at 30°C in a YEPD medium for 14–16 h (the so-called overnight culture) and transferred, in a certain amount,

[1] Corresponding author. E-mail: eugene@sifibr.irk.ru

0026-2617/03/7205-0548$25.00 © 2003 MAIK "Nauka / Interperiodica"

to a fresh YEPD (or YEPGal, in which glucose was substituted by an equivalent amount of galactose) medium. The medium was incubated for 3–4 h, during which the culture density increased to about 10^7 cells/ml (OD = 0.3–0.4).

Cell respiration was measured at 30°C in the YEPD medium (1.4 ml) using a Clark-type oxygen electrode. Sodium malonate was added to the cell suspension at final concentrations of 1, 5, and 10 mM. The respiration rate was expressed in nmol O_2 consumed by 10^7 cells in one minute.

To study the effect of malonate on the thermotolerance of yeast cells, it was added at a concentration of 5 mM to test tubes containing 1 ml of the cell suspension prepared using either a YEPD or YEPGal medium. After exposing cells to heat shock at 45 or 50°C over a specified period, the cell suspension was cooled, appropriately diluted, and plated onto YEPD agar. The number of colonies grown on the plates was determined after 24–48 h of incubation at 30°C. The survival rate was calculated as a percent of colonies grown from heat-exposed cells relative to the number of colonies grown from unexposed yeast cells.

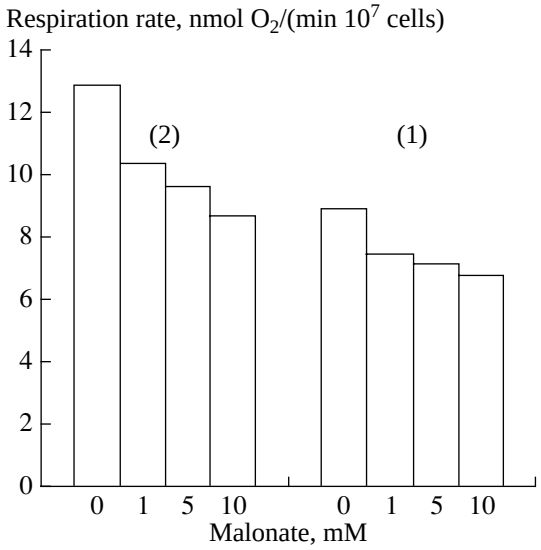

Fig. 1. The effect of malonate (0, 1, 5, and 10 mM) on the respiration rate of (1) *R. rubra* and (2) *S. cerevisiae* cells, which were grown at 30°C in a YEPD medium to the logarithmic growth phase.

RESULTS

Sodium malonate added to yeast cell suspensions inhibited cell respiration to a considerably lower degree than azide or cyanide [5, 6]. Specifically, 10 mM malonate inhibited the respiration of *S. cerevisiae* and *R. rubra* cells by 33 and 24%, respectively (Fig. 1). Such low inhibitory activity of malonate can be accounted for by the fact that the transfer of electrons to the ubiquinone pool of the yeast respiratory chain is provided not only by succinate dehydrogenase (complex II of the respiratory chain), but also by the rotenone-insensitive dehydrogenase oxidizing external NADH and, at least in the case of the yeast *R. rubra*, the rotenone-sensitive NADH dehydrogenase (complex I). There is evidence that the respiratory chain of the yeast *S. cerevisiae* lacks complex I [10].

According to earlier observations [5], heat shock at 45°C does not significantly affect the survival of *R. rubra* cells. For this reason, the effect of malonate on the thermotolerance of *R. rubra* cells was studied by exposing them to heat shock at 50°C, which exerted a profound lethal effect on *R. rubra* cells. The addition of 5 mM malonate considerably diminished the thermotolerance of these cells, as is evident from the tenfold smaller number of colonies grown from yeast cells exposed to 15-min heat shock in the presence of malonate (Fig. 2).

Unlike *R. rubra* cells, *S. cerevisiae* cells were fairly sensitive to heat shock at 45°C. In contrast to the case with *R. rubra*, the addition of 5 mM malonate to *S. cerevisiae* cells exposed to heat shock in a YEPD medium did not reduce the survival rate of these cells either at 45°C (Fig. 3a) or at 50°C (Fig. 3b). Moreover, as can be seen from Fig. 3b, malonate exerted a slight protective effect on *S. cerevisiae* cells exposed to heat shock at 50°C for 2 min, although already after the next 2 min of exposure, the protective effect of malonate was statistically insignificant. The protective effect of malonate resembles the protective effect of azide on the thermotolerance of *S. cerevisiae* in glucose-containing media [5, 6].

Thus, malonate diminished the thermotolerance of the nonfermentative yeast *R. rubra* but did not influence (and sometimes even slightly increased) the thermotolerance of the glucose-fermenting yeast *S. cerevisiae*. Bearing in mind our earlier finding that sodium azide exerts opposite effects on the survival of *S. cerevisiae* cells incubated in the presence of glucose and galactose [6], we performed some experiments on the thermotolerance of these cells in the presence of galactose.

In accordance with earlier observations [6], *S. cerevisiae* cells incubated in the presence of galactose (YEPGal medium) are more thermotolerant than the cells incubated in the presence of glucose (YEPD medium). Unlike the case with heat shock in the presence of glucose (Fig. 3), malonate significantly decreased the thermotolerance of *S. cerevisiae* cells exposed to heat shock in the presence of galactose (Fig. 4). Therefore, when *S. cerevisiae* cells utilize a carbon source (in the given case, galactose) predominantly through oxidative phosphorylation, malonate impairs the functioning of mitochondria and, as in the case of *R. rubra*, enhances the damaging effect of heat shock on yeast cells.

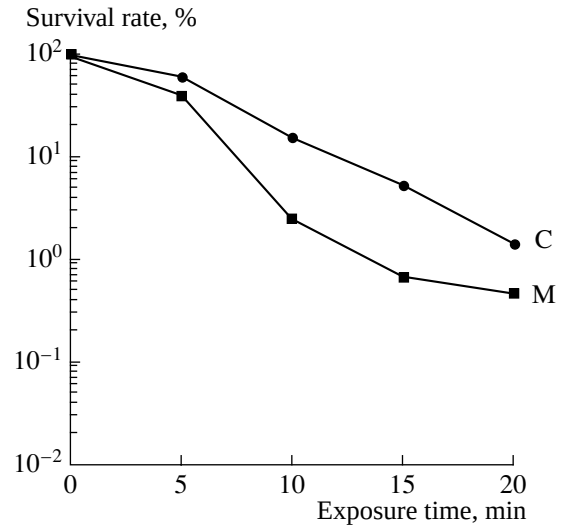

Fig. 2. The effect of 5 mM malonate (M) on the thermotolerance of glucose-grown *R. rubra* cells, which were grown at 30°C in a YEPD medium to the logarithmic growth phase and then exposed to heat shock at 50°C. C is the control.

DISCUSSION

The data obtained show that if yeast cells utilize glucose through oxidative phosphorylation, the impairment of the functioning of mitochondria by the succinate dehydrogenase inhibitor malonate augments the lethal action of heat shock. But if yeast cells utilize glucose through fermentation, malonate does not influence and sometimes even improves their thermotolerance. Similar data were obtained earlier for the other respiratory inhibitors, azide and cyanide [5, 6]. Therefore, there is a correlation between the thermotolerance of yeast cells and the functional activity of mitochondria, provided that these cells satisfy their energy requirements through oxidative phosphorylation.

This inference agrees with the information available in the literature that the repair of heat-induced damage with molecular chaperones is an energy-dependent process [11] and that ATP deficiency in cells may enhance the damaging effect of heat shock [12]. Until now, there have been no reports as to the involvement of mitochondria in the protection of yeast cells from heat shock. Moreover, based on the observation that the responses of the wild-type *S. cerevisiae* and its respiratory mutant to heat shock were similar, it was considered that yeast thermotolerance does not require fully functional mitochondria [13, 14]. The experimental results presented in this and earlier papers [5, 6] give grounds to believe that mitochondria are not important in the protection from heat shock of *S. cerevisiae* cells that obtain energy through fermentation, but become very important when these cells obtain energy through oxidative phosphorylation.

The beneficial effect of azide [5, 6] and malonate (Fig. 3b) on the thermotolerance of the glucose-grown

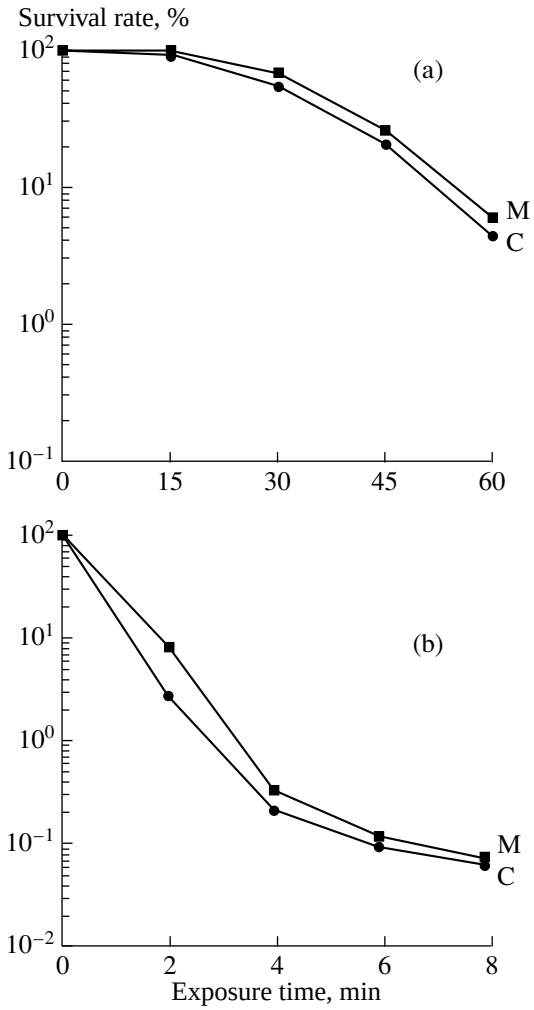

Fig. 3. The effect of 5 mM malonate (M) on the thermotolerance of glucose-grown *S. cerevisiae* cells, which were grown at 30°C in a YEPD medium to the logarithmic growth phase and then exposed to heat shock at (a) 45 and (b) 50°C. C is the control.

S. cerevisiae cells is to be understood in depth, although some relevant speculations can be made even now. It is known that anaerobic *S. cerevisiae* cells are much more tolerant to heat shock than are aerobic yeast cells [7] and that elevated temperatures enhance the respiration of yeast cells under aerobic conditions [8, 15–17]. It was suggested that heat shock may raise the concentration of reactive oxygen species in cells due to enhanced respiratory activity [8, 17], which is in agreement with the fact that the main source of ROS in cells is the mitochondrial respiratory chain. It is unlikely that the protective effect of azide and malonate is merely due to the inhibition of respiration, since cyanide is a very potent respiratory inhibitor, but it does not exhibit protective activity [5]. Nevertheless, we believe that azide and malonate can prevent the excessive accumulation of ROS in cells by interfering with the normal work of mitochondria by some way or another. It should be

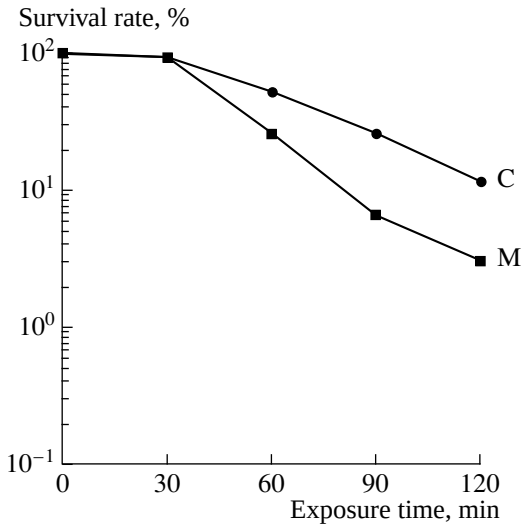

Fig. 4. The effect of 5 mM malonate (M) on the thermotolerance of galactose-grown *S. cerevisiae* cells, which were grown at 30°C in a YEPGal medium to the logarithmic growth phase and then exposed to heat shock at 45°C. C is control.

noted in this regard that malonate inhibits the formation of hydrogen peroxide in succinate-oxidizing mitochondria [18].

It is likely that the role of mitochondria in cell thermotolerance is twofold. On the one hand, fully functional mitochondria are necessary for the reactivation of heat-damaged cellular structures and the maintenance of cell survival. On the other hand, such mitochondria, generating ROS in response to heat shock, are harmful to cells. In view of this, it is not surprising that yeast cells exposed to heat shock die not only because of protein denaturation [11] but also because of the toxic action of ROS [7].

Let us discuss now the survival curve of *S. cerevisiae* cells exposed to heat shock at 50°C (Fig. 3b). As can easily be seen, this curve is biphasic, the first phase (exposure for 0–4 min) being characterized by a steeper decrease in the cell survival rate than the second stage (exposure for 4–8 min). Such a shape of the survival curve was interpreted by van Uden as an indication that the cell population contains a subpopulation of more thermotolerant cells [19]. However, Schenberg-Frascino and Moustacchi [20] showed that there are no genetically stable subpopulations of yeast cells in the population exposed to heat shock at 52°C. This is evident from the fact that the cells that survived long-term exposure to 52°C and then were again exposed to heat shock exhibited the same biphasic survival curve. In our opinion, the biphasic survival curve is due to the existence of different mechanisms of cell damage. Indeed, according to our earlier observations [17], the cell respiration of heat-exposed (50°C) cells drastically increases within the first 2 min and then rapidly falls, reaching (and then becoming below) the initial level already after 4 min. The decrease in respiration is not directly associated with a fall in the number of viable cells, since nonviable cells can remain metabolically active for some time [21, 22]. Thus, the first phase of the survival curve, which is characterized by a rapid decrease in the survival rate of yeast cells, corresponds to a higher level of their respiration. In view of this, we suggest that the enhanced respiration of yeast cells during the first phase of cell response to heat shock is one of the damaging factors. This explains why the protective effect of the respiratory inhibitor malonate on the yeast cells exposed to heat shock at 50°C is profound only within 2 min (Fig. 3b).

REFERENCES

1. Kappeli, O., Regulation of Carbon Metabolism in *Saccharomyces cerevisiae* and Related Yeasts, *Adv. Microb. Physiol.*, 1986, vol. 28, pp. 181–209.
2. De Deken, R.H., The Crabtree Effect: A Regulatory System in Yeast, *J. Gen. Microbiol.*, 1966, vol. 44, no. 2, pp. 149–156.
3. Kreger van Rij, N.J.W., *Debaryomyces* Lodder et Kreger–van Rij nom. conserv., *The Yeast: A Taxonomic Study*, Lodder, J., Ed., Amsterdam: North-Holland Publ., 1970, pp. 129–156.
4. Phaff, H.J. and Ahearn, D.G., *Rhodotorula* Harrison, *The Yeast: A Taxonomic Study*, Lodder, J., Ed., Amsterdam: North-Holland Publ., 1970, pp. 1187–1223.
5. Rikhvanov, E.G., Varakina, N.N., Rusaleva, T.M., Rachenko, E.I., and Voinikov, V.K., The Effect of the Cytochrome Oxidase Inhibitors on the Thermotolerance of Yeasts, *Mikrobiologiya*, 2003, vol. 72, no. 2, pp. 174–179.
6. Rikhvanov, E.G., Varakina, N.N., Rusaleva, T.M., Rachenko, E.I., and Voinikov, V.K., The Effect of Sodium Azide on the Thermotolerance of the Yeasts *Saccharomyces cerevisiae* and *Candida albicans*, *Mikrobiologiya*, 2002, vol. 71, no. 6, pp. 768–772.
7. Davidson, J.F., Whyte, B., Bissinger, P.H., and Schiestl, R.H., Oxidative Stress Is Involved in Heat-Induced Cell Death in *Saccharomyces cerevisiae*, *Proc. Natl. Acad. Sci. USA*, 1996, vol. 93, no. 10, pp. 5116–5121.
8. Sugiyama, K., Izawa, S., and Inoue, Y., The Yap1p-Dependent Induction of Glutathione Synthesis in Heat Shock Response of *Saccharomyces cerevisiae*, *J. Biol. Chem.*, 2000, vol. 275, no. 20, pp. 15535–15540.
9. Slater, E.C., Application of Inhibitors and Uncouplers for a Study of Oxidative Phosphorylation, *Methods Enzymol.*, 1967, vol. 10, pp. 48–57.
10. Joseph-Horne, T., Hollomon, D.W., and Wood, P.M., Fungal Respiration: A Fusion of Standard and Alternative Components, *Biochim. Biophys. Acta*, 2001, vol. 1504, no. 2/3, pp. 179–195.
11. Parsell, D.A. and Lindquist, S., The Function of Heat-Shock Proteins in Stress Tolerance: Degradation and Reactivation of Damaged Proteins, *Annu. Rev. Genet.*, 1993, vol. 27, pp. 437–496.
12. Nguyen, V.T. and Bensaude, O., Increased Thermal Aggregation of Proteins in ATP-depleted Mammalian Cells, *Eur. J. Biochem.*, 1994, vol. 220, no. 1, pp. 239–246.

13. Mitchel, R.E. and Morrison, D.P., Assessment of the Role of Oxygen and Mitochondria in Heat Shock Induction of Radiation and Thermal Resistance in *Saccharomyces cerevisiae*, *Radiat. Res.*, 1983, vol. 96, no. 1, pp. 113–117.
14. Weitzel, G., Pilatus, U., and Rensing, L., The Cytoplasmic pH, ATP Content, and Total Protein Synthesis Rate during Heat-Shock Protein Inducing Treatments in Yeast, *Exp. Cell Res.*, 1987, vol. 170, no. 1, pp. 64–79.
15. Pozmogova, I.N., The Effect of Submaximal Temperatures on Thermotolerant Yeasts, *Mikrobiologiya*, 1976, vol. 45, pp. 97–99.
16. Patriarca, E.J. and Maresca, B., Acquired Thermotolerance Following Heat Shock Protein Synthesis Prevents Impairment of Mitochondrial ATPase Activity at Elevated Temperatures in *Saccharomyces cerevisiae*, *Exp. Cell Res.*, 1990, vol. 190, no. 1, pp. 57–64.
17. Rikhvanov, E.G., Varakina, N.N., Rusaleva, T.M., Rachenko, E.I., Kiseleva, V.A., and Voinikov, V.K., Heat Shock–induced Changes in the Respiration of the Yeast *Saccharomyces cerevisiae*, *Mikrobiologiya*, 2001, vol. 70, no. 4, pp. 531–535.
18. Korshunov, S.S., Skulachev, V.P., and Starkov, A.A., High Protonic Potential Actuates a Mechanism of Production of Reactive Oxygen Species in Mitochondria, *FEBS Lett.*, 1997, vol. 416, no. 1, pp. 15–18.
19. van Uden, N., Temperature Profiles of Yeasts, *Adv. Microb. Physiol.*, 1984, vol. 25, pp. 195–251.
20. Schenberg-Frascino, A. and Moustacchi, E., Lethal and Mutagenic Effects of Elevated Temperature on Haploid Yeast: I. Variations in Sensitivity during the Cell Cycle, *Mol. Gen. Genet.*, 1972, vol. 115, no. 3, pp. 243–257.
21. Hanninen, A.-L., Simola, M., Saris, N., and Makarow, M., The Cytoplasmic Chaperone Hsp104 Is Required for Conformational Repair of Heat-denatured Proteins in the Yeast Endoplasmic Reticulum, *Mol. Cell. Biol.*, 1999, vol. 10, no. 11, pp. 3623–3632.
22. Sozinov, D.Yu., Rikhvanov, E.G., Varakina, N.N., Rachenko, E.I., and Voinikov, V.K., The Effect of the Postshock Temperature on the Survival of *Debaryomyces vanriji*, *Fiziol. Rast.* (Moscow), 1999, vol. 46, no. 2, pp. 276–281.