Volume 220, number 1, 113-115 FEB 04998 August 1987

Rapid changes of heat and desiccation tolerance correlated with changes of trehalose content in *Saccharomyces cerevisiae* cells subjected to temperature shifts

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Received 22 June 1987

The trehalose content of exponentially growing *Saccharomyces cerevisiae* cells rapidly increased in response to a temperature shift from 27 to 40°C and decreased again when the temperature was shifted back from 40 to 27°C. These changes were closely correlated with increases and decreases in the thermotolerance and desiccation tolerance of the cells. Our results support the hypothesis that trehalose functions as a protectant against heat and desiccation.

Heat shock; Trehalose; Thermotolerance; Desiccation tolerance; (*Saccharomyces cerevisiae*)

1. INTRODUCTION

The non-reducing disaccharide, trehalose, occurs in large amounts in the spores of yeast and many other fungi [1]. In anhydrobiotic organisms, trehalose has been proposed to serve as a protectant against desiccation stress [2]. This is based on evidence that membranes can be desiccated in the presence of trehalose without losing their structural and functional integrity [2-4].

Yeast cells growing exponentially on glucose contain only little trehalose [1]. However, enormous amounts of this disaccharide are accumulated in response to a heat shock (Hottiger et al., submitted). Here, we demonstrate that accumulation or mobilization of trehalose in response to temperature shifts is highly correlated with an increase or decrease in desiccation tolerance. In addition, the trehalose content is also correlated with the thermotolerance of the cells. Our results indicate that trehalose has a protective role not only in desiccation stress but also in heat stress.

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2. EXPERIMENTAL

2.1. *Organism and cultivation*

Saccharomyces cerevisiae (strain C276, a/α, see [5]) was cultivated on a synthetic medium containing 2% glucose, 20 mM NH_4Cl, salts and vitamins as in [6]. All experiments were performed during the first exponential growth phase at cell densities $\leq 2 \times 10^7$ cells/ml.

2.2. *Induction of trehalose accumulation and trehalose measurements*

Accumulation of trehalose was induced by transferring cultures from 27°C (normal growth temperature) to a water bath at 40°C (heat shock treatment). For trehalose measurements, cells (10 ml) were collected by filtration (Whatman GF/C), thoroughly washed with cold (0°C) bidistilled water and extracted with 1 ml cold 5% (w/v) trichloroacetic acid. Trehalose in the acid extracts was measured as described [5].

2.3. *Determination of desiccation tolerance*

The ability of cells to survive desiccation was tested as follows: 5-ml samples were collected on membrane filters (Millipore, type HA, 0.45 μm) and thoroughly washed with cold bidistilled water.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

When the water had been sucked off, the filters were placed in petri dishes and stored overnight in a desiccator partly filled with silica gel. The cells were then suspended in an appropriate amount of sterile water and briefly sonicated to minimize clumping. Aliquots (200 μl) were plated in triplicate on potato dextrose agar (Oxoid, prepared according to the instructions of the manufacturer). Colonies formed within 2–3 days were used to calculate the percentage of survivors. Total cell numbers were determined in a hemocytometer, immediately before plating.

2.4. *Determination of thermotolerance*

Thermotolerance was tested according to Hall [7], but cells were heated to 50.4°C (8 min) instead of 52°C. Samples were plated in triplicate on potato dextrose agar (see above).

2.5. *Protein*

Protein was determined according to Peterson [8]. Bovine serum albumin served as the standard.

3. RESULTS

During exponential growth on glucose, cells of *S. cerevisiae* contained only traces of trehalose. However, large amounts of this disaccharide were accumulated in response to a heat shock. When the heat-shocked cells were allowed to recover at 27°C, their trehalose pool was rapidly depleted (fig.1B).

The ability of the cells to survive desiccation increased about 6-fold upon incubation at 40°C for $\geq$ 60 min. When the heat-shocked cells were transferred back to 27°C, desiccation tolerance was lost within less than 1 h (fig.1A). The trehalose content of the cells and their desiccation tolerance increased and decreased with almost identical kinetics during heat shock and subsequent recovery (fig.1A,B). A similar correlation was established between the trehalose content and the thermotolerance of the cells (fig.2A,B). These results suggest that trehalose protects yeast from

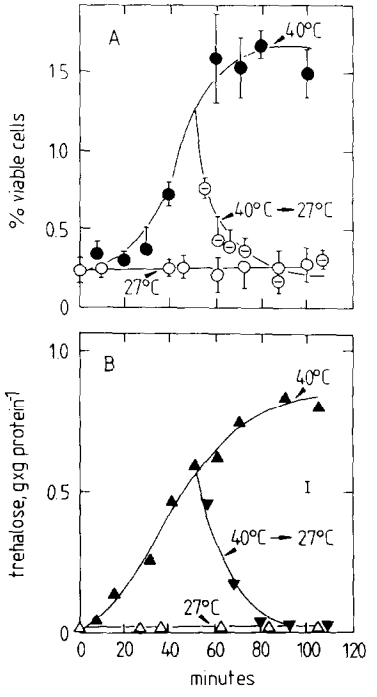

Fig.1. Changes of desiccation tolerance (A) and trehalose content (B) of *S. cerevisiae* upon heat shock (●,▲) and subsequent recovery (⊖,▼). (○,△) Controls maintained at 27°C. Values represent means of 3 measurements. Bars in (A) indicate SE; the bar on the right in (B) the maximal SE.

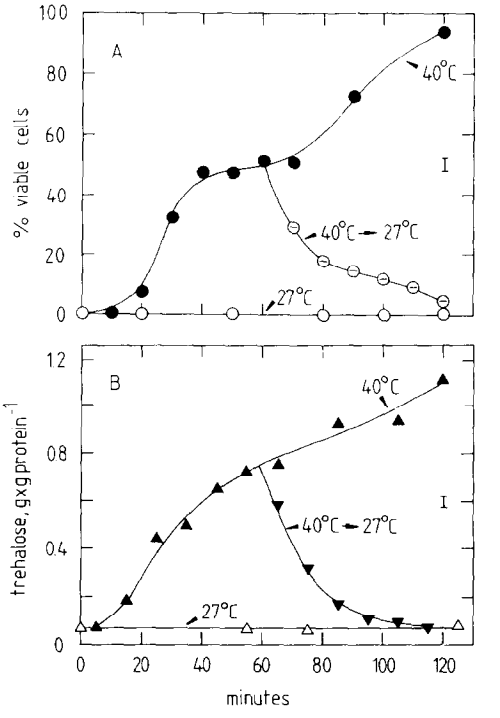

Fig.2. Changes of thermotolerance (A) and trehalose content (B) of *S. cerevisiae* upon heat shock and subsequent recovery. Symbols as in fig.1. Values represent means of 3 determinations. Bars on the right indicate maximal SE.

thermal injury as well as from damage during desiccation and rehydration.

4. DISCUSSION

The accumulation of trehalose in fungal spores has been considered a form of energy storage [1]. In addition, it may represent a protection against desiccation [2]. In fact, recent work with *Phycomyces blakesleeanus* spores indicates that this could be its only function: During germination, these spores do not make use of the accumulated trehalose as an energy source but rapidly metabolize it to glycerol which is released into the medium [9].

In yeast, the trehalose present in resting cells is rapidly metabolized upon transfer of the cells into growth medium. Growing yeast cells normally contain very little trehalose [1]. However, when subjected to a heat treatment, growing cells of *S. cerevisiae* accumulate enormous amounts of this disaccharide (up to 1 g per g protein, see fig.1B). It was tested whether trehalose accumulation might have a protective function. We initially examined the desiccation tolerance of yeast cells subjected to temperature shifts (fig.1A). Our results show an excellent correlation between the changes in desiccation tolerance and trehalose content of the cells, indicating that trehalose provides protection against desiccation. Note that desiccation and rehydration in distilled water were used as a test for desiccation tolerance, a rather harsh treatment which resulted in a relatively small fraction of survivors.

It makes sense, from an ecological point of view, that heat treatment increases the desiccation tolerance of yeast cells: In the natural habitat of fungi, heat would often announce a subsequent period of dryness. However, it is well known that yeast and many other organisms adapt to heat also in a more direct sense: Heat shock generally leads to a marked increase in thermotolerance [10]. Consequently, we also tested the influence of temperature shifts on the thermotolerance of growing yeast cells and compared it with changes in their trehalose content (fig.2). Again, we found a remarkable correlation, suggesting that trehalose might protect yeast cells against heat stress as well. At present, it is mostly thought that thermotolerance is mediated by heat shock proteins [10]. However, it is unlikely that heat shock proteins are responsible for the short-term changes in thermotolerance observed here. In particular, the rapid decay of thermotolerance in response to the shift from 40 to 27°C argues against this possibility, since the turnover of heat shock proteins is very slow [11]. However, a close look at the kinetics (fig.2A) indicates that a small degree of thermotolerance persists after the phase of rapid decay. It is likely that this part of thermotolerance is due to a protecting system different from trehalose which reacts more slowly on temperature shifts. Heat shock proteins would be obvious candidates for this system.

In conclusion, our results indicate that heat-induced trehalose accumulation protects growing yeast cells against both heat and desiccation stress. While the increase in desiccation tolerance may be due to the stabilizing effect of trehalose on membranes, it remains to be shown by what mechanism trehalose could increase thermotolerance.

ACKNOWLEDGEMENT

This work was supported by the Swiss National Science Foundation.

REFERENCES

[1] Thevelein, J.M. (1984) Microbiol. Rev. 48, 42-59.
[2] Crowe, J.H., Crowe, L.M. and Chapman, D. (1984) Science 223, 701-703.
[3] Crowe, L.M., Crowe, J.H., Rudolph, A., Womersley, C.W. and Appel, L. (1985) Arch. Biochem. Biophys. 242, 240-247.
[4] Martin, M.C., Diaz, L.A., Manzanal, M.B. and Hardisson, C. (1986) FEMS Microbiol. Lett. 35, 49-54.
[5] Lillie, S.H. and Pringle, J.R. (1980) J. Bacteriol. 143, 1384-1394.
[6] Keller, F., Schellenberg, M. and Wiemken, A. (1982) Arch. Microbiol. 131, 298-301.
[7] Hall, B.G. (1983) J. Bacteriol. 156, 1363-1365.
[8] Peterson, G.L. (1977) Anal. Biochem. 83, 346-356.
[9] Van Laere, A., François, A., Overloop, K., Verbeke, M. and Van Gerven, L. (1987) J. Gen. Microbiol. 133, 239-245.
[10] Neidhardt, F.C., Van Bogelen, R.A. and Vaughn, V. (1984) Annu. Rev. Genet. 18, 295-329.
[11] Cavicchioli, R. and Watson, K. (1986) FEBS Lett. 207, 149-152.