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Short communication

Dehydration of yeast: Changes in the intracellular content of Hsp70 family proteins

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ABSTRACT

Yeast is known to experience in natural and industrial conditions cycles of dehydration–rehydration. Several molecular mechanisms can be triggered in response to this and other environmental stressors and to rescue yeast cells of the cytotoxic effect. Since heat shock proteins constitute one of the most important systems of the response to stress we studied whether the pre-induced major stress protein, Hsp70, can cope with yeast cell drying. To induce Hsp70 expression the cells of two yeast species, *Saccharomyces cerevisiae* and *Debaryomyces hansenii*, were subjected to non-lethal heat shock. It was found that during yeast culture growth Hsp70 accumulation occurred at the exponential growth phase, and there was no marked change in the protein level at the stationary phase both in aerobic and anaerobic conditions. Interestingly, dehydration of sensitive to this kind of stress *S. cerevisiae* grown in anaerobic conditions led to the increase of Hsp70 expression; to our knowledge this finding was presented for the first time. Dehydration of yeast taken from the stationary growth phase did not cause the induction of Hsp70 expression. Irrespective of the inducer, Hsp70 did not rescue yeast cells from dehydration stress damages. This result well coincides with data of other groups found that Hsp70 in yeast possesses chaperonic activity, and the latter does not impact to an increase in protective power of the protein demonstrated in many other organisms.

1. Introduction

Yeasts as many other types of microorganisms can be subjected to significant changes of humidity in nature. As the result they can undergo multiple cycles of dehydration and subsequent rehydration in their life. During evolution they worked out a variety of mechanisms which help to maintain their viability at their transfer into "non-active" state of anhydrobiosis. This state is characterized by a transient and reversible reduction of metabolism and also by a variety of changes at biochemical and functional levels [1]. The latter include condensation of chromatin, separation by membranes of rather big parts of nucleus and damaged areas of cytoplasm [2–4], synthesis of trehalose and polyols [5–7], stabilization of molecular organization of intracellular membranes [8], maintenance of redox homeostasis [9] and other.

Heat shock proteins belonging to Hsp70 family are established to be the ubiquitous stress-responsive system in all living organisms. The accumulation of Hsp70 signals that a cell or tissue respond to an environmental or xenobiotic harmful factor, and in most cases the increase of Hsp70 expression renders cells more resistant to repetitive stressors. Intracellular functions of Hsp70 are based on its chaperonic activity that implies assembly, folding, intracellular localization, secretion, and degradation of cellular polypeptides [10–12]. Protective power of Hsp70 thought to be linked to its chaperonic activity is proved by studies on hundreds cell and animal models.

The genome of *Saccharomyces cerevisiae* yeast contains 14 genes comprising multigene Hsp70 family proteins [13]. This protein family includes mitochondrial proteins Ssc1 and Ssc1p [14–17], cytosolic proteins Ssa1, Ssa1p, Ssa2 and Ssa4p which accumulate in cell nucleus during yeast starvation [18]. As in other organism in yeast Hsp70 chaperones facilitate endoplasmic reticulum-associated degradation of "defective" proteins [19]. It is known also that the cytosolic yeast Hsp70 supervises proteins involved in the response to stress and protein synthesis [20]. Loss of mitochondrial Hsp70 (Ssc1p) function causes aggregation of mitochondrial polypeptides in yeast cells [21]. *S. cerevisiae* cells with Hsp70 knockout demonstrate abnormal nuclear distribution and aberrant microtubule formation in M-phase [22]. A few factors inducing Hsp70 expression in yeast include heat shock and oxidative stress;

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it is also noteworthy that high amount of the chaperone was found in cells subjected to deuterium oxide or genetically resistant to low temperatures [23]. The expression and role of Hsp70 in conditions of dehydration and rehydration remains unexplored. The aim of this study was to analyze the possible function of pre-established Hsp70 in cells of two yeast strains subjected to drying as well as to understand if dehydration stress itself leads to the synthesis of Hsp70.

2. Materials and methods

2.1. Yeast strains and cultivation conditions

In this study we used yeast *S. cerevisiae* 14 (Collection of the Laboratory of Cell Biology, Institute of Microbiology and Biotechnology, University of Latvia) and *Debaryomyces hansenii* (generous gift from Prof. L. Adler, Geteborg University, Sweden). The latter strain was earlier found to be significantly more resistant to dehydration. Yeast cells were cultivated in 750 ml flasks at 30 °C using shaker (180 rpm) for aerobic conditions (*S. cerevisiae* and *D. hansenii*) and without shaking with a great excess of nutrient medium for anaerobic conditions (only *S. cerevisiae*). Nutrient medium contained (in g l^{-1}): $MgSO_4$ 0.7; NaCl 0.5; $(NH_4)_2SO_4$ 3.7; KH_2PO_4 1.0; K_2HPO_4 0.13; molasses 43 (till final concentration of glucose 20 g l^{-1}). pH value of nutrient medium was adjusted to pH 5.0 using H_2SO_4.

2.2. Biomass harvesting and dehydration

Yeast cells at the exponential phase (for yeast grown in aerobic conditions) and stationary phase (for yeast grown both in aerobic and anaerobic conditions) were collected. To establish time points for the harvesting of the biomass (data not present) direct counting of cell amount in Goryaev chamber and spectrophotometric determination of suspension's optical density at 600 nm were performed. Harvested biomass was washed and compressed with the aid of vacuum filtration unit. A part of yeast biomass was used in further experiments as "native" counterpart, the second part was dehydrated by convective method at 30 °C to residual humidity of 8–10%, and the third portion of biomass was used for the experiments on heat shock. This part was subjected to heat shock and also was dehydrated till the residual humidity of 8–10%. Biomass relative humidity was measured of its weight after drying at 105 °C during 48 h.

2.3. Determination of cells viability

Viability of native and dehydrated cells was measured with the help of fluorescent microscopy using specific probe primuline [24]. The use of this method gives the possibility to reveal live organisms in which only cell wall fluoresces and dead yeast which have bright green fluorescence of the whole cell.

2.4. Heat shock

Compressed biomass was put in 250 ml flask. 75 ml of pre-heated till 42 °C filtered cultural liquid was added to the flask. Procedure of heat shock was made at 42 °C during 30 min. After heat stress yeast cells were transferred to fresh nutrient medium in which they were kept 1 h at 30 °C.

2.5. Quantification of Hsp70 by immunoblotting

To measure Hsp70 content the method of Western blotting was employed using protocol of Towbin et al. [25]. Briefly, yeast cells were subjected to disintegration in 0.1 M K-potassium buffer (pH 7.0) with glass beads (diameter 300 mkm) during 10 min at 4000 rpm with refrigeration using the disintegrator SCP-100-MRE, Innomed-Konsult AB, Sweden. The samples of total protein extract from disintegrated yeast cells and were mixed with sodium dodecylsulfate (SDS) and 2-mercaptoethanol to give final concentration 2% and 15 mM, respectively. Equal amounts of the total protein, 50 μg, were applied onto lanes of 10% polyacrylamide gel. Electrophoresis was performed with a voltage gradient of 5 V cm^{-1} and currency 30 mA per gel slab. After the electrophoresis protein bands were transferred onto Immobilon nitrocellulose membrane with the aid of the semi-dry blotting apparatus (GE Healthcare, Russia) according to standard protocol [25]. The bands of Hsp70 were stained with the use of SPA-822 monoclonal antibody known to recognize inducible component of the yeast Hsp70 family (StressGen, Canada).

3. Results

The major goal of this study was to elucidate whether heat precondition accompanying with the accumulation of Hsp70 stress protein can protect yeast cells from the deleterious effect of dehydration as well as to understand if dehydration stress leads to the synthesis of Hsp70 proteins in yeast. To establish the conditions of Hsp70 accumulation we studied the protein level in control and stressed yeast cells.

The analysis of Hsp70 expression during the *S. cerevisiae* growth was performed in samples taken each 4 h after the cells had been seeded in nutrient medium. This study was performed with the aid of Western blotting and showed that the level of Hsp70 was strongly elevated during first 8 h after inoculation that corresponded to exponential growth phase (Fig. 1). Twelve hours after inoculation the level of Hsp70 began to decline and 12 h later the signal fully disappeared. The yeast entered stationary phase of growth at time point 18 h after seeding, and the reduction of Hsp70 level revealed that despite a strong lowering of cellular metabolism the protein is subjected to proteolysis. Thus, the highest level of Hsp70 can be attained in the middle of exponential phase and this point was selected for further experiments on pre-conditional stress designed to increase Hsp70 amount in cells.

Since dehydration by itself can induce stress response, we measured Hsp70 amount in *S. cerevisiae* cells grown in aerobic conditions, taken at the exponential growth phase and subjected to dehydration. It was found that drying led to a complete reduction of Hsp70 level (Fig. 2A). Viability of these cells was also found to be at very low level—14.8 ± 1.15% (Fig. 2C). Dehydration of the same yeast taken at the stationary phase did not cause expression of Hsp70 (Fig. 2A). In these experiments viability of dehydrated cells was 65.4 ± 0.65% that is ordinary value for this yeast grown and dehydrated in "standard" conditions in our previous studies of anhydrobiosis [1]. Finally, dehydration of yeast grown in anaerobic conditions and taken from stationary growth phase led to the accumulation synthesis of Hsp70 family proteins (Fig. 2B). It is necessary to mention that this yeast was extremely sensitive to dehydration and the maximal viability did not exceed 1%.

To check whether the same response to stress is typical for various yeast species, we studied profile of Hsp70 expression in *D. hansenii* cells that are extremely resistant to dehydration [26]. Similar to *S. cerevisae* these cells were found to contain Hsp70 at the exponential phase of growth and not at the stationary phase (Fig. 3A). Dehydration of yeast *D. hansenii* taken from exponential growth phase led to the reduction of Hsp70 content (Fig. 3A). As suggested the viability of dehydrated *D. hansenii* remained high enough in contrast with *S. cerevisiae*, and comprised 55–60%. Lastly dehydration of *D. hansenii* cells taken from stationary growth

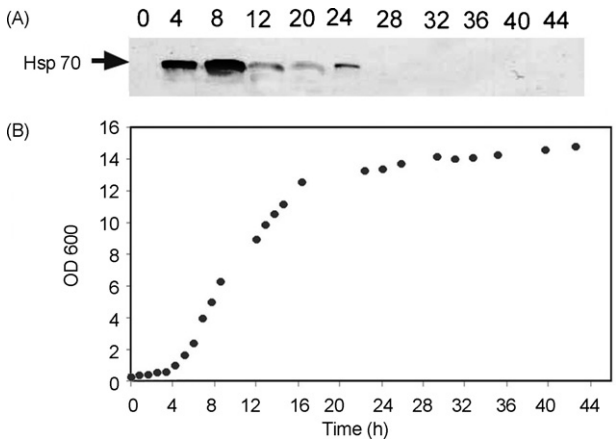

Fig. 1. Hsp70 protein content in the cells of *Saccharomyces cerevisiae* during its growth in aerobic conditions: (A) Hsp70 protein content at different phases of culture growth; (B) yeast culture growth curve.