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Role of Selenium Supplementation and Heat Stress on Trehalose and Glutathione Content in *Saccharomyces cerevisiae*

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Abstract

The role of selenium (Se) supplementation on glutathione, a potent intracellular redox buffer, and trehalose, a well-known stress protectant molecule, was studied. The amount of glutathione decreased significantly while that of trehalose showed a minor decrease in the cells grown in Se-supplemented medium. After heat shock, glutathione content diminished further, whereas that of trehalose increased significantly in control and Se-supplemented cells. These findings suggest the importance of trehalose as an antioxidant molecule.

Index Entries: Antioxidants; heat shock; oxidative stress; selenium supplementation; yeast.

Introduction

A major question in biology is, how do cells cope with rapid changes in their microenvironment, such as exposure to elevated temperature, heavy metals, toxins, and oxidants? It has become clear that all organisms share a common molecular response that includes changes in pattern of gene expression and elevated synthesis of a family of heat-shock or stress-induced proteins *(1,2)*. On the other hand, there is convincing evidence to suggest that these are probably not the principal factors following induction of a rapid resistance to lethal stress *(3)*. Other physiologic factors that confer thermotolerance include induction of respiratory deficiency and trehalose biosynthesis *(4,5)*. Heat stress has also been implicated in the generation of oxidative stress, and in the maintenance of cellular redox state, which, in turn, is protected by both nonenzymatic and enzymatic

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mechanisms *(6,7)*. Owing to industrial pollution and acid rain, living organisms are being constantly challenged by various metal stresses. Metals also serve as essential cofactors for a variety of enzymatic reactions and play structural and functional roles in cellular metabolism.

Microbes when grown in a medium supplemented with salts of trace elements often take up these elements by active transport through plasma membrane or by physicochemical absorption on the cell surface. Metal ions are known to confer tolerance to sudden heat shock. As implied by the structural similarity of selenium (Se) to sulfur, Se may be expected to replace sulfur in various biosynthetic reactions of the living systems. Recently, the role of Se as an antioxidant has generated a wide interest in it. Apart from being established as an essential component of selenoprotein, glutathione peroxidase, Se is increasingly being recognized as a versatile anticarcinogen against a number of spontaneous, chemically, and virally induced tumors. Although Se enrichment practices have become very popular in higher organisms with inorganic sources as well as by Se-enriched yeasts, not many biochemical studies have been done in the yeast *(8)*. Microorganisms, especially yeasts, are very useful models for laboratory-based metal toxicity studies. The purpose of the present work was to study the effect of Se supplementation on levels of trehalose and glutathione, two important molecules known as indicators of general stress response in *Saccharomyces cerevisiae*.

Materials and Methods

Chemicals

Yeast extract, bactopeptone, agar powder, and dextrose were obtained from Himedia Labs (Mumbai, India). Bovine serum albumin (BSA), Coomassie brilliant blue G-250, glutathione, DTNB, and sodium borohydride were from Sigma (St. Louis, MO). All other chemicals were of analytical grade and purchased from local companies.

Organism and Culture Conditions

S. cerevisiae RS 924, generously provided by Prof. Ramon Serrano (Valencia, Spain), was maintained at 4°C on YPDA (2% dextrose, 1% yeast extract, 2% peptone, and 2% agar). Filter-sterilized sodium selenite solution was added to the preautoclaved YPD (pH 5.5) medium to a final concentration of 0.1 m*M*. The cells were grown in Erlenmeyer flasks with a liquid-to-air volume ratio of 1:5 at 200 rpm and 30°C. Cells (mid–log phase) were harvested by centrifuging at 2000*g* for 5 min and washed once with distilled water and twice with phosphate-buffered saline (PBS) (200 m*M* phosphate containing 600 m*M* KCl, pH 6.0).

Harvested cells were suspended aseptically in YPD and heated rapidly to 48°C in a water bath for 30 min. Samples were shaken during the treatment. Stress was relieved by transferring 0.1-mL portions to an ice

bath, and 10 mL of PBS (0.1 *M* phosphate containing 600 m*M* KCl, pH 6.0) was added at room temperature and analyzed for viability.

Measurement of Viability

Appropriate dilutions containing approx 200 cells were spread in triplicates on YPDA medium. Plates were incubated for 3 d at 30°C. The percentage of survivors was determined by comparison with viable counts of unstressed yeast samples.

Extraction and Assay of Trehalose

Extraction of trehalose was performed according to the method of Lillie and Pringle *(9)* as described previously *(10)*. Briefly, the cell pellet was quickly chilled on an ice bath and vortexed with a threefold volume of 500 m*M* trichloroactic acid and then extracted for 60 min at room temperature. After centrifugation, the supernatant was collected and the extraction process was repeated with the pellet. Combined supernatants from two extractions were assayed for trehalose by the anthrone procedure *(11)*. Because this procedure detects both trehalose and glucose, the amount of trehalose was determined after subtracting the value of glucose or any other reducing sugar(s) quantified by the *o*-toluidine method *(12)*.

Estimation of Glutathione

A known quantity of cells was homogenized in 10% (w/v) chilled TCA. The homogenate was centrifuged at 10,000*g* for 10 min, and the clear supernatant was used to estimate reduced as well as total glutathione. The methodology of Beutler et al. *(13)* was followed for the estimation of reduced glutathione. The content of glutathione in yeast cells was calculated from the standard curve. The total glutathione content of the yeast cell homogenate was estimated by the method of Habeeb *(14)* after reduction of GSSG to GSH with sodium borohydride.

Estimation of Protein

The pellet obtained after trehalose extraction was solubilized by boiling for 5 min with 2.0 mL of 0.1 *M* NaOH. The solution was neutralized with 0.1 *M* HCl. The clear supernatant obtained after centrifuging at 5000*g* for 10 min was used for protein estimation by the Bradford *(15)* procedure using BSA as the standard.

Results and Discussion

Cells possess both enzymatic and nonenzymatic defense systems to protect their cellular constituents against oxidative stress and to maintain an optimal cellular redox state. Among the nonenzymatic ones, the best known example is GSH. This molecule is thought to act as a radical scavenger with redox-active sulfydryl group reacting with oxidants to convert

it into oxidized form (i.e., GSSG). The content of GSH was determined in cells grown in the presence of Se and control (without Se), both before and after heat shock; the data are given in Table 1. The total content of glutathione decreased from 21.80 ± 0.21 μg/mg of protein in the control cells to 9.75 ± 0.11 μg/mg of protein in Se-supplemented cells. Similarly, after heat shock, the values of glutathione in both the control and Se-supplemented cells reduced to nearly half their value without heat shock. This decrease in glutathione content, as observed in our experiments during Se supplementation, might be an adaptation, in which cells did not need components of the glutathione redox cycle. The role of Se as a cellular antioxidant defense system is unquestionable *(16)*.

It is well known that heat-shock treatment exerts oxidative stress on the cells and, therefore, cellular levels of GSH were estimated (Table 1). As expected, their levels decreased significantly in both cases (control and Se-supplemented cells). The viability after heat shock was much less, about 50% in control and 40% in Se-supplemented cells. This difference could be attributable to the decreased GSH content of Se-treated cells. Indeed, GSH concentration has been directly linked to thermal tolerance of cells *(17,18)*. In contrast to the findings of Chang et al. *(19)*, who reported induction of thermotolerance on exposure of microorganisms to a variety of metal oxides, our results did not show any significant difference between control and Se-supplemented cells. Se, being analogous to sulfur, has been covalently incorporated into proteins as selenocysteine and selenomethionine, whereas the present results are supported by the observation of Kohrola et al. *(20)*, who detected various pools of selenoglutathione and selenodiglutathione. Furthermore, Se enrichment has been known to increase glutathione peroxidase activity, which, in turn, utilizes cellular GSH *(16)*. Therefore, the decrease in natural glutathione pool during Se supplementation could have resulted in the synthesis of a new pool of selenoglutathione and selenodiglutathione that was not detected by standard colorimetric procedures.

The decrease in glutathione pools may be explained by increased glutathione-*S*- transferase and γ-glutamyl transpeptidase activities, which are well known to take up GSH as a cofactor. In addition, the use of GSH as a redox buffer to membrane-bound sulfydryls could be another reason for these decreased GSH and GSSG levels. Further, Krezel and Bal *(21)* have shown recently that metal ions form stable complexes with reduced and oxidized glutathione, which is responsible for delivering a variety of metal ions to apoproteins. Metals have also been detoxified by chelation to GSH and then transported to vacuoles by adenosine triphosphate binding cassette transporters *(22)*.

The relative amount of oxidized glutathione (GSSG) decreased from 51.6% in control cells to 36.0% in Se-supplemented cells after heat shock, further supporting our finding that during Se enrichment, GSH is not used as a major water-soluble chain-breaking antioxidant. As a result, the GSH/GSSG ratio increased from 0.94 ± 0.06 in unsupplemented cells to

Table 1
Effect of Se Supplementation on Glutathione Level in Cells of *S. cerevisiae* Before and After Heat Shock[a]

Group	Total glutathione, T (μg/mg protein)	Reduced glutathione, R (μg/mg protein)	Oxidized glutathione ([$T - R$], μg/mg protein)	Ratio reduced:oxidized
Control	21.80 ± 0.21	10.55 ± 0.14	11.25 ± 0.19	0.94 ± 0.06
Se treated	9.75 ± 0.11	6.24 ± 0.25	3.51 ± 0.17	1.78 ± 0.09
Control after heat shock	10.76 ± 0.28	5.54 ± 0.23	5.22 ± 0.18	1.06 ± 0.12
Se treated after heat shock	4.98 ± 0.25	3.46 ± 0.12	1.52 ± 0.09	2.28 ± 0.34

[a]Results are the mean ± SD of three independent experiments performed in triplicate.

Table 2
Effect of Se Supplementation on Trehalose Level in Cells of *S. cerevisiae* Before and After Heat Shock[a]

Group	Trehalose content		Viability (%)
	µg/mg protein	ng/cell	
Control	55 ± 0.46	0.13 ± 0.004	100
Se enriched	40 ± 0.39	0.08 ± 0.001	100
Control after heat shock	110 ± 2.48	6.11 ± 0.069	50
Se enriched after heat shock	140 ± 3.56	7.00 ± 0.092	40

[a]Results are the mean ± SD of three independent determinations in triplicate.

1.78 ± 0.09 in cells treated with Se, lending support to the aforementioned findings *(16)*.

GSH depletion and consequent alterations in GSH/GSSG ratio can affect the activity of enzymes of carbohydrate metabolism *(23)*. Hence, it is worthwhile to measure the amount of trehalose under the conditions of Se supplementation. Trehalose and glycogen are generally regarded as two main reserve carbohydrates in yeast, and their levels vary during physiologic conditions *(24)*. On the other hand, however, several lines of evidence suggest that trehalose primarily functions not only as a reserve carbohydrate but also as a highly efficient molecule to maintain structural integrity of cells under several adverse environmental conditions *(3,25)*.

Trehalose content of the cells was measured after Se supplementation and heat shock. The level of trehalose in control cells increased from 55 to 110 µg/mg of protein after heat stress (Table 2). Similarly, Se-treated cells the level of trehalose increased from 40 to 140 µg/mg of protein after shock. These results corroborate the previous observation of Hottiger et al. *(25,26)* that trehalose content in the cells could be an accurate marker for the thermotolerant state of cells, suggesting that trehalose may act as a thermoprotectant. These findings show that after heat stress trehalose content in control cells and Se-treated cells increased 2.0 and 3.5 times, respectively. Thus, in Se-treated cells there was a significant increase in trehalose levels after heat stress compared with control cells. This may be owing to a double shock that the Se-treated cells received. The first one was owing to the Se supplementation and the second to the heat stress.

These results suggest that trehalose acts not only as a thermoprotectant, but also as a general stress protectant that protects the cells against heat stress as well as other types of stress. It does seem likely that a certain threshold level of trehalose is vital for resistance to environmental stresses, including oxidative stress *(27–29)*. Therefore, trehalose may have a role as an antioxidant at lower glutathione concentration.

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