

Effect of Reserve Carbohydrates on Oxidative Stress in Yeast *Saccharomyces cerevisiae* Y6210

Smita Kanwal, R.K. Saharan, A. Mahmood and S.C. Sharma
Department of Biochemistry, Panjab University, Chandigarh,-160014 India

Abstract: The aim of this study is investigate the role of reserve carbohydrates, trehalose and glycogen in DEM (Diethyl Maleate) induced oxidative stress in yeast *Saccharomyces cerevisiae* Y6210. Trehalose and glycogen accumulated in *Saccharomyces cerevisiae*, when growth conditions deteriorate. Yeast cells were subjected to oxidative stress for different time periods (0, 30, 60 and 120 min) to evaluate the role of trehalose, glycogen and trehalase. There was no change in the level of trehalose while the content of glycogen increased during the oxidative stress. The time dependent modulation in the activities of trehalase and invertase was observed under oxidative stress. It has been suggested that glycogen serving as a protectant during oxidative stress not trehalose.

Key words: Diethyl maleate, glycogen, invertase, oxidative stress, trehalase, trehalose

INTRODUCTION

In nature, as well as under laboratory conditions or in industrial processes, yeast is subjected to different adverse environmental situations, such as osmotic, thermal and oxidative stresses. Under severe stress conditions, however, the fermentation ability of yeast is limited. Yeast cells also need to acquire a variety of stress-adaptation mechanisms, for their survival and growth (Belloch *et al.*, 2008). One such adaptation is to accumulate reserve carbohydrates such as glycogen and trehalose, when nutritional conditions deteriorate (Lillie and Pringle, 1980). Initially believed as a storage factors, trehalose and glycogen were later implicated in other roles as well. Trehalose and glycogen accumulate not only upon carbon starvation but also under other stress conditions such as nitrogen or sulfur starvation, heat shock, or osmotic stress (Hottiger *et al.*, 1987, 1989; Eleutherio *et al.*, 1993; De Vergilio *et al.*, 1994; Parrou *et al.*, 1997; Mahmud *et al.*, 2009) led to the suggestion that they can act as stress protectants. Trehalose synthesis is catalyzed by the trehalose synthase enzyme complex with UDP-glucose and glucose-6-phosphate used as substrates. Trehalose can be degraded by two trehalases, acid and neutral, which hydrolyzes the disaccharide to yield two molecules of glucose (Londesborough and Varimo, 1984; Nwaka and Holzer, 1997). The vacuolar acid trehalase (Ath1p) (Destruelle *et al.*, 1995; Alizadeh and Klionsky, 1996) is constitutively active with its pH optimum at 4.5 and has been classically considered not to be regulated by stress. As glucose is the fundamental unit for trehalose and glycogen, so we want to investigate how stress affect the levels of these two glucose stores and to integrate the regulation of reserve carbohydrates metabolism in the survival of yeast cells. In the yeast

Saccharomyces cerevisiae, some studies have also shown that trehalose and its hydrolysis may play an important physiological role during the life cycle of the cell. Thus, we examined the activity of neutral trehalase during oxidative stress. Moreover, the neutral trehalase (Nth1p) is responsible for the intracellular mobilization and/or recycling of trehalose (Nwaka *et al.*, 1995 a, b; Parrou *et al.* 1997; Kopp *et al.*, 1993; Nwaka and Holzer, 1998) and also regulated by the general stress-response pathway through the interaction of Msn2/Msn4 transcriptional factors with stress-response elements (STREs) present in the NTH1 gene promoter (Zahringer *et al.*, 1997; Zähringer *et al.*, 2000). In present study, the activity of invertase was also investigated in response to oxidative stress.

MATERIALS AND METHODS

The experiments for the study were carried out during the academic session 2009-2010 at Yeast Stress Response Study Laboratory of Biochemistry Dept, Panjab University at Chandigarh, India.

Organism and culture condition: Yeast strain, *Saccharomyces cerevisiae*, Y 6210 was maintained at 4°C on YPDA (2% dextrose, 1% yeast extract, 2% peptone and 2% agar). The cells were grown in YPD media (pH 5.5) in Erlenmeyer flasks with liquid-to-air volume ratio of 1:5 at 200 rpm and 30°C for 15-17 h.

Oxidative stress condition: An YPD culture was inoculated and grown till mid log phase. 5 mM DEM (Diethyl Maleate) was added to the cell culture at 30°C to induce oxidative stress by depleting glutathione content

(McNutt-Scott and Harris, 1998). Samples were collected at interval of 30 min for 2 h.

Trehalose extraction and determination: Samples taken from the cultures grown at 30°C were washed twice with ice-cold water to remove external glucose and media. Then suspended in the distilled water and transferred to a water bath (95°C) for 10 min to denature and precipitate proteins. After centrifugation at 20,000 x g for 15 min. Trehalose and glycogen was estimated in supernatant by the Anthrone method as described previously (Wyatt and Kalf, 1957; Jagdale and Grewal, 2003). The pellet was used for the protein estimation.

Assay of disaccharidases: Invertase (EC 3.2.1.26), and trehalase (EC 3.2.1.28) activities were assayed by measuring D-glucose liberated from the respective disaccharides, using a glucose-oxidase-peroxidase system kit. The method used was a modification of that described by Dahlqvist (1964). Specific activity is expressed as Units of glucose per milligram of protein. One enzyme unit is defined as the amount of enzyme required to hydrolyze 1 mmol of substrate to the product per min under standard assay conditions.

Protein estimation: The pellet obtained after trehalose and glycogen extraction was solubilised by boiling for 5 min with 2.0 mL of 0.1 M NaOH. The clear supernatant was used for protein estimation by Lowry method (Lowry *et al.*, 1951) using bovine serum albumin as standard.

Statistical analysis: Statistically significant differences were determined by using the one way analysis of variance (ANOVA). Values with $p < 0.05$ were considered as statistically significant. Analyses were performed using GraphPad Prism v.5.00.288 for Windows (GraphPad Software, San Diego, CA).

RESULTS

Trehalose content: Yeast cells were cultured for 17 h to reach log phase under optimum conditions. Cells were subjected to oxidative stress by adding 5 mM DEM

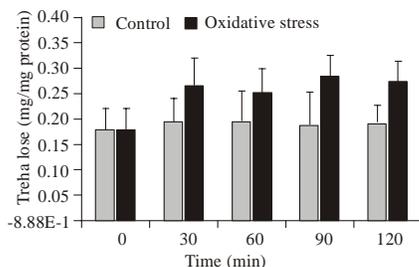


Fig. 1: Effect of oxidative stress on trehalose content of *Saccharomyces cerevisiae* on different time intervals. The value is mean of three independent experiments

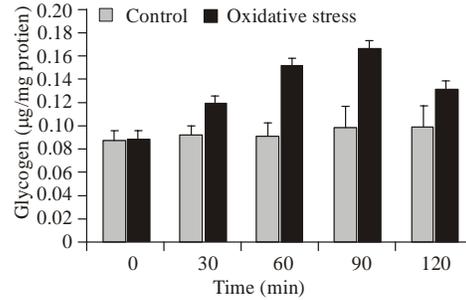


Fig. 2: Effect of oxidative stress on glycogen content of *Saccharomyces cerevisiae* on different time intervals. The value is mean of three independent experiments

Table 1: Effect of oxidative stress induced by 5 mM DEM (Diethyl Maleate) on invertase activity in *Saccharomyces cerevisiae*

Time (min)	Control	Oxidative stress
0	4.53±0.11	
30	4.55±0.11	5.63±0.24**
60	4.91±0.22	4.07±0.14**
90	4.65±0.26	4.19±0.18
120	5.23±0.17	3.68±0.23***

Values represent means ± SD of three independent experiments. *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$

(Diethyl maleate) for 2 h. Samples were collected at 30, 60, 90 and 120 min to determine the trehalose content. There was no significant difference in trehalose content of cells grown in oxidative stressed and non-stress conditions. The trehalose content of stressed yeast cells was about 1.5 times higher than control cells throughout the experiment (Fig. 1). The level of trehalose was enhanced after 30 min of treatment from 0.195 ± 0.046 to 0.266 ± 0.052 mg/mg protein. After 60 min, DEM (5 mM) treated cells maintained high content of trehalose as compared with untreated cells (Fig. 1).

Glycogen content: The content of glycogen increased in oxidative stressed cells during the course of experiment compare with controls. The level of glycogen was increased by 27.43 and 31.54% at 30 and 120 min after treatment with DEM (diethyl maleate) in yeast cells. However, the amount of glycogen increased 0.092 ± 0.010 in control to 0.150 ± 0.006 and 0.099 ± 0.017 µg/mg in control to 0.165 ± 0.007 after 60 min and 90 min, respectively, in stressed yeast cells (Fig. 2).

Invertase activity: As shown in Table 1, the activity of invertase increased by 23.74% at 30 min of exposure to oxidative stress. The invertase activity decreased gradually from 5.63 ± 0.24 to 3.68 ± 0.23 units/mg protein during the course of the experiment after addition of DEM (5 mM) in exponential phase culture as compared to unstressed cells.

Trehalase activity: The trehalase activity was also measured in the yeast cells after addition of 5 mM diethyl

Table 2: Effect of oxidative stress induced by 5 mM DEM (Diethyl Maleate) on trehalase activity in *Saccharomyces cerevisiae*

Time(min)	Control	Oxidative stress
0	4.14±0.46	
30	4.12±0.25	4.20±0.37
60	4.20±0.08	5.47±0.18***
90	4.88±0.15	4.45±0.35
120	5.28±0.36	7.65±0.29***

Values represent means±SD of three independent experiments.*: p≤0.05; **: p≤0.01; ***: p≤0.001

maleate (DEM) in the medium. The activity of trehalase was increased by 30 and 45% at 60 and 120 min of treatment with DEM, respectively as compared to control cells (Table 2). There was no change in trehalase activity at 30 and 90 min under this condition.

DISCUSSION

Glycogen and trehalose are the two major reserve carbohydrates in the yeast *Saccharomyces cerevisiae* and can represent up to 25 % of the dry cell mass, depending on the environmental conditions (Lillie and Pringle, 1980). Trehalose is widely distributed in various organisms, including bacteria, fungi, plants, insects and invertebrates (Nwaka, and Holzer, 1998), in which it accumulates dramatically during stationary phase, heat shock, and oxidative stress (Hounsa *et al.*, 1998; Herdeiro *et al.*, 2006). Since GSH is an important antioxidant and helps to maintain a strong reducing environment in the cell, its oxidation or depletion should mimic some of the effects of exerting oxidative challenge. Thus, the GSH state of the cell was modulated in order to study the oxidative stress response. GSH can be oxidized by diamide treatment or depleted by conjugation with diethyl maleate (Boyland and Chasseaud, 1967). In present study, there was no significant difference in trehalose content of cells grown in presence of 5 mM DEM stressed and non-stress conditions. However, the activity of neutral trehalase increased after 1 h of oxidative stress. It implicates that increase in trehalase activity help to degrade the trehalose into glucose. This may help to maintain the need of carbon and energy during stress condition.

Similar result was observed by Pedreño *et al.* (2002) that studied the oxidative stress induced by H₂O₂ in ath1 and nth1 deletion mutants of *Saccharomyces cerevisiae*. The low concentrations of H₂O₂ induced no significant level of trehalose but marked activity of Nth1p. When yeast cells were challenged with NaCl (0.3-0.5 M) or H₂O₂ (0.4-1.0 mM), very low or even no accumulation of glycogen and trehalose is found in spite of transcriptional activation of the genes involved in their metabolism (Parrou *et al.*, 1997; Zahringer *et al.*, 1997). A net deposition of reserve carbohydrates is nevertheless observed after hinder the degradation pathways by deletion of GPH1 and NTH1. This indicates that a major

outcome of this coordinated stress-induced gene activation is to stimulate a recycling of glycogen and trehalose molecules (Zahringer *et al.*, 1997; Godon *et al.*, 1998). This may explain the accumulation of glycogen in yeast cells subjected to oxidative stress. These results confer the role of glycogen in the control of glycolytic flux under stress for survival of the cell. Furthermore, this strain displayed an adaptive response to oxidative stress that was independent of intracellular trehalose synthesis. The ability of *S. cerevisiae* to ferment high sucrose concentrations (including those in sweet bread doughs) is related inversely to activity of invertase, which hydrolyzes sucrose (Myers *et al.*, 1997). The activity of the invertase was also examined during oxidative stress to find out the tolerance of yeast strain to high concentrations of sucrose that is desirable for sweet dough fermentation (Nagodawithana and Trivedi, 1990; Atfield, 1997; Higgins *et al.*, 2001) and also osmotic stress (due to high sugar concentration).

The activity of invertase increased at 30 min after treatment with DEM thereafter decreases in the activity of yeast cell. This shows that decrease in the level of glutathione also affect the activity of invertase.

CONCLUSION

Present data suggest that trehalose storage in yeast *Saccharomyces cerevisiae* Y6210 is not an essential protectant in cell defense against oxidative challenge. However, oxidative stress enhances the production of glycogen to protect the yeast cells.

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