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Trehalose accumulates in *Saccharomyces cerevisiae* during exposure to agents that induce heat shock response

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The storage disaccharide, trehalose, is accumulated in yeast during a temperature shift from 30 to 45°C. The response peaks at 90 min and is transient since levels of trehalose decline rapidly in cells returned to 30°C. Storage of trehalose is inhibited when cells are incubated in the presence of acridine orange or ethidium bromide prior to and during temperature shift, suggesting a requirement for de novo RNA synthesis. Accumulation of trehalose occurs when cells are exposed to either ethanol, copper sulphate or hydrogen peroxide at 30°C, indicating that the phenomenon may be a general response to physiological stress. Parallels are drawn between the trehalose accumulation response and the heat shock response in yeast.

Trehalose; Reserve carbohydrate; Stress response; Heat shock; *(Saccharomyces cerevisiae)*

1. INTRODUCTION

The reserve carbohydrate trehalose (α-D-glucopyranosyl α-D glucopyranoside) occurs commonly in nature. It is found in organisms as diverse as bacteria, yeast and other fungi, algae and lower plants, insects and invertebrates [1,2]. Originally trehalose was thought to function, along with glycogen, as an energy reserve in yeast [3–5]. The disaccharide has also been proposed to act as a regulator in glycolytic flux [6]. Recent reports indicate that trehalose also functions both as a protectant during freezing and a membrane preservative during desiccation [7–10].

Large amounts of trehalose are accumulated in *Saccharomyces cerevisiae* when it is shifted rapidly to temperatures exceeding the normal growth range [11]. It is well known that exposure of organisms to environmental stresses such as heat, heavy metals, oxidants and organic solvents, induces the expression of a set of genes called heat shock genes [12,13]. Molecular analyses indicate that these genes are highly conserved across taxa ranging from bacteria to humans and including yeast. The functional significance of the heat shock genes remains unclear, although it seems likely that their products serve to protect cells from toxic effects of stress [13]. Only a few of the bacterial heat shock proteins have been linked to functions [12] and with the exception of ubiquitin [14], this author knows of no other heat shock proteins in eucaryotes that have been functionally defined.

Given that trehalose acts as a cellular protectant under harsh conditions and that it is accumulated in cells shifted to higher temperatures, it seems plausible that the disaccharide represents a stress response product. This paper reports experiments designed to test the effects of different inducers of the heat shock response on trehalose levels in yeast. Results indicate that various environmental insults are capable of causing trehalose accumulation and that heat induced storage of the disaccharide requires de novo RNA synthesis.

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2. MATERIALS AND METHODS

2.1. *Yeast strains and culture conditions*

Saccharomyces cerevisiae strains AH22 *a leu-2 his-4 can-1* and ATCC no.46815 *αtrp* were mated using standard genetic techniques [15]. The resulting prototrophic diploid was designated HS-1. Strains were grown routinely by shaking at 160 rpm and 30°C in GYP medium, which was 2% (w/v) D-glucose, 1% (w/v) bacteriological peptone, 0.5% (w/v) yeast extract and 0.3% (w/v) potassium dihydrogen orthophosphate.

2.2. *Stressful treatments*

Cultures were prepared by inoculating 5×10^3 cells into 50 ml GYP in 250 ml Erlenmeyer flasks. They were grown to a density of $1-2 \times 10^7$ cells/ml (logarithmic fermentation phase). At this point flasks were transferred to a shaking water bath preset at the desired heat shock temperature. For stressing with ethanol, copper sulphate or hydrogen peroxide, cells were grown to a density of $1-2 \times 10^7$/ml. The desired concentration of chemical was then added and incubation with shaking at 30°C continued. After stressing cultures for the desired period, flasks were placed on ice. For testing the effects of acridine orange (ACO) or ethidium bromide (EBr) cells were grown to $1-2 \times 10^7$/ml and drugs added separately 20 min prior to application of stress.

2.3. *Extraction and assay of trehalose*

Chilled cells were washed 3 times in equal volumes of cold distilled water by centrifugation at $3000 \times g$ and 4°C for 5 min. Cell pellets were sampled for dry weight contents and extracted twice with 500 mM trichloroacetic acid as described [5]. Extracts were assayed for trehalose using the anthrone assay [16]. Control experiments showed that none of the chemicals used interfered with the assays.

Samples were analysed by thin-layer chromatography to ensure that trehalose was the material being assayed. They were spotted onto Whatman 20×20 cm silica gel-60 thin-layer chromatography plates, which were then double-developed using butanol/acetone/0.1 M potassium dihydrogen orthophosphate, pH 6.0 (4:5:1) as the solvent. After allowing plates to air-dry, samples were detected using the diphenylamine/aniline/phosphoric acid procedure [17]. Trehalose standards were distinguished readily from glucose. Analysis of trichloroacetic acid extracts (not shown) indicated that those giving anthrone-positive reactions also produced spots migrating to the same position as trehalose standards. No other spots were detected in these samples. Extracts giving anthrone-negative reactions failed to produce visible spots on chromatograms.

3. RESULTS

When growing yeast cells are shifted from 30°C to 45°C they accumulate significant amounts of trehalose (table 1, fig.1). The storage of trehalose occurs rapidly under these conditions, reaching a plateau after 90 min and showing little decline over 240 min (fig.1). However, if cells are returned to

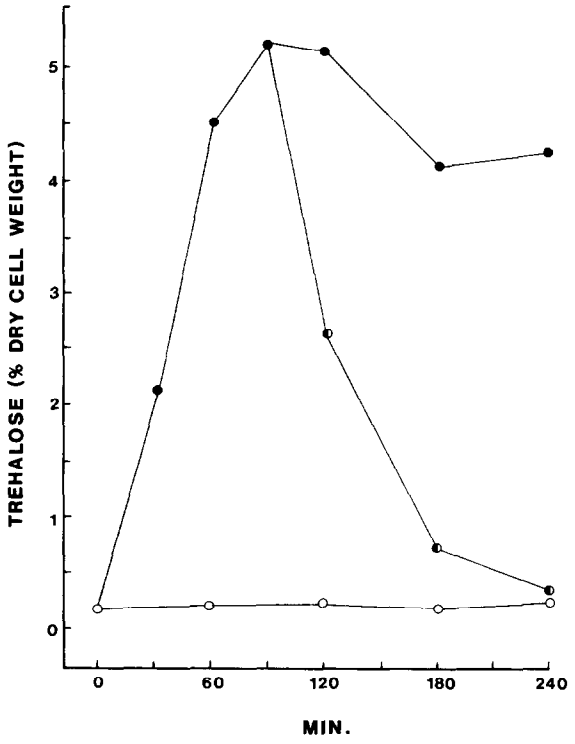

Fig.1. Accumulation of trehalose by *Saccharomyces cerevisiae* during temperature shift. Strain HS-1 was grown in GYP at 30°C to a density of $1-2 \times 10^7$ cells/ml and subjected to various temperature shifts. Control cells incubated at 30°C (○); cells shifted from 30°C to 45°C (●); cells shifted from 30°C to 45°C then returned to 30°C (◐).

30°C following a 90 min temperature shock at 45°C, there is a rapid decline in the level of trehalose (fig.1).

Requirement for de novo RNA synthesis in trehalose accumulation during heat shock was tested by incubating growing cells in the presence of ACO or EBr prior to and during temperature shift. Treatment of cells with 5 mM ACO or 2.5 mM EBr resulted in significant inhibition of trehalose accumulation (table 1), suggesting that RNA synthesis is required for the response.

To test whether agents other than heat can induce trehalose storage, either 1.6 M ethanol, 5 mM copper sulphate or 5 mM hydrogen peroxide was added to cells growing at 30°C. All three agents induced significant amounts of trehalose accumulation in strain HS-1 (fig.2). Haploid strains also accumulated trehalose under these conditions although their responses were muted by toxicity of the agents (not shown). Irreversible toxicity resulting in cell death, may explain the eventual fall in trehalose contents of diploid strain HS-1 when exposed to copper sulphate or hydrogen peroxide for prolonged periods (fig.2). There was a 44% drop in yield of HS-1 after 360 min in the presence of copper sulphate (relative to the yield at time of addition of the chemical). Similarly, there was a 60% fall in relative yield of HS-1 exposed to hydrogen peroxide for 240 min. In contrast the relative yield of HS-1 increased by 100% during the 360 min incubation in the presence of ethanol, and there was a steady increase in trehalose content of these samples (fig.2).

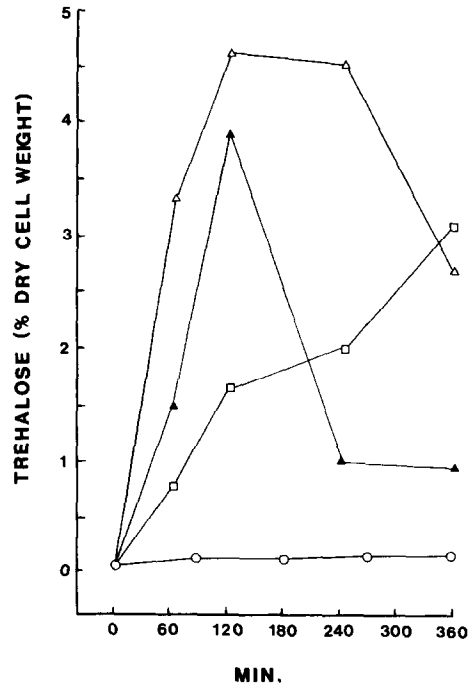

Fig.2. Accumulation of trehalose by *Saccharomyces cerevisiae* during exposure to noxious chemicals. Chemical agents were added to strain HS-1 growing at a density of 1–2 × 10^7/ml in GYP at 30°C. Control, untreated cells (○); plus 1.6 M ethanol (□); plus 5 mM copper sulphate (△); plus 5 mM hydrogen peroxide (▲).

Table 1

Accumulation of trehalose in strains of *Saccharomyces cerevisiae* exposed to temperature shift

Conditions	Trehalose (% dry cell wt)		
Strain:	HS-1	AH22	ATCC 46815
1. Grown at 30°C	0.22	0.20	0.48
2. Grown at 30°C and shifted to 45°C for 90 min	6.02	5.14	5.19
3. As 2, but 5 mM ACO added 20 min before shift to 45°C	0.32	0.41	0.33
4. As 2, but 2.5 mM EBr added 20 min before shift to 45°C	1.50	0.67	1.67

4. DISCUSSION

Trehalose is accumulated when growing yeast cells are shifted to higher temperatures (table 1, fig.1). Similar observations have been reported by others [11,18]. This seems to be a general response of *Saccharomyces cerevisiae*, since all other strains that have been tested behave in a similar manner (unpublished). The accumulation of trehalose seems to be somewhat transient in that levels decline rapidly in cells returned to normal growth temperatures (fig.1); see also [18]. The pattern of heat induced trehalose accumulation and diminution shows an interesting parallel to the heat shock

response in yeast. Synthesis of heat shock proteins occurs rapidly in cells shifted to higher temperatures [13]. Moreover, the response seems to be transient because the rate of heat shock protein synthesis reaches a plateau after about 90 min and declines when cells are returned to normal growth temperatures [13,19,20].

The disappearance of trehalose on return of heat shocked cells to 30°C (fig.1) may be explained by the fact that cells do not accumulate trehalose and in fact mobilise any existing deposit of the disaccharide when growing on glucose under normal physiological conditions [2]. There is evidence to suggest that the biosynthesis and breakdown of trehalose by trehalose-6-phosphate synthase and trehalase, respectively, is regulated by cyclic AMP-dependent protein phosphorylation. Thus, trehalose-6-phosphate synthase is phosphorylated and inactive, whereas trehalase is phosphorylated and active, during normal growth on glucose when cyclic AMP levels are high [21–23]. Given these findings the return of heat shocked yeast to normal growth temperature and conditions in glucose-containing medium could be expected to provide the correct physiological state for mobilisation and rapid diminution of stored trehalose.

Accumulation of trehalose was inhibited by incubation of cells in the presence of either ACO or EBr prior to heat shock (table 1). Both of these agents inhibit RNA synthesis [24]. The apparent requirement of heat induced trehalose storage for RNA synthesis resembles the heat shock response, which is controlled at the level of transcription in yeast [13]. Moreover, the accumulation of trehalose in cells treated with ethanol, copper sulphate or hydrogen peroxide (fig.2) indicates that this may be a general response to stress, which resembles the heat shock response still further. There are other similarities between trehalose accumulation and the synthesis of heat shock proteins. For example, trehalose is stored in large quantities by cells entering stationary phase or those starved of nutrients such as nitrogen, phosphorus or sulphur [5]. Trehalose is also stored in ascospores and is required for germination [2,25]. There is evidence that heat shock proteins are produced in yeast during growth arrest at the G1 phase [26]. Heat shock proteins are also found in cultures undergoing transition to stationary phase, and during sporulation [27,28]. Thus trehalose and heat shock proteins appear to share the property of functioning in normal cell cycle and development as well as in recovery from stress.

Finally, there are intriguing parallels between trehalose metabolism and function and the heat shock response. Whilst the similarities are circumstantial it is nevertheless plausible that the disaccharide could represent a product of the heat shock response in yeast.

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