Alcohols lower the threshold temperature for the maximal activation of a heat shock expression vector in the yeast *Saccharomyces cerevisiae*

Brendan P. G. Curran and S. A. Khalawan

Author for correspondence: Brendan P. G. Curran. Fax: +44 81 983 0973.

School of Biological Sciences, Queen Mary and Westfield College, Mile End Road, London E1 4NS, UK

When the yeast *Saccharomyces cerevisiae* is exposed to elevated growth temperatures, genes containing a heat shock element (HSE) in their promoters are activated. This study demonstrates that alcohols lower the temperature required for the maximal activation of such a promoter and that the concentration of alcohol required decreases as its hydrophobicity increases. A similar correlation has been found between the members of this alcohol series and their effect on a range of membrane functions. Our results therefore indicate that perturbation of the cell membrane may play a role in the heat induced activation of this HSE-containing promoter.

Keywords: *Saccharomyces cerevisiae*, heat shock response, heat shock element, cell membrane, alcohols

INTRODUCTION

All living cells examined to date produce an evolutionarily conserved group of proteins, the heat shock proteins, when they are exposed to a sudden, sublethal increase in their growth temperature. Some or all of these proteins are also induced when the cells are exposed to other stressing agents such as alcohol, heavy metals and anoxia (for reviews see Mager & Ferreira, 1993; Lindquist, 1986). The presence of a short DNA sequence, the heat shock element (HSE), in the promoters of these genes determines the regulation of this expression (Pelham, 1982). A heat shock transcription factor (HSTF) can bind to a promoter containing a HSE and subsequently activate the gene (Sorger & Pelham, 1988; Wiederrecht *et al.*, 1988). In yeast, the level of heat shock gene activation is determined by the level of HSTF phosphorylation; increased phosphorylation induces increased gene expression (Sorger & Pelham, 1988). However, the mechanism(s) responsible for transducing heat stress into an intracellular message resulting in HSTF phosphorylation, has (have) yet to be elucidated (for a review see Sorger, 1991).

The classical view of the heat shock response (HSR) is that stressing agents induce protein denaturation, with a resultant induction of the stress response. At the mol-

ecular level, HSTF appears to be maintained in a repressed state in the absence of heat shock. It may be that heat stress causes negative regulatory proteins to dissociate from the HSTF, or heat stress may simply induce conformational changes in the HSTF itself (see Sorger, 1991). An alternative view is that the plasma membrane becomes leaky as a consequence of heat stress and that changes in intracellular pH may somehow be responsible for heat shock gene activation (Coote *et al.*, 1991). These two models are not mutually exclusive because protein conformation is pH sensitive, but if heat stress is transduced into an intracellular signal at the level of the cell membrane, then molecules (such as alcohols) that interact with the membrane might be expected to alter the cell's HSR profile.

In this paper we demonstrate that the temperature for the maximal induction of a heat-shock-inducible expression vector decreases in the presence of alcohol, and that the concentration of alcohol required to achieve this effect is inversely related to the alcohol's hydrophobicity. These results implicate a perturbation of the cell membrane in the heat induced activation of this heat shock sensitive promoter and support the view that thermal stress may be transduced into a cellular signal at the level of the cell membrane.

METHODS

Strains. A haploid strain of *Saccharomyces cerevisiae*, DBY747 *MATa leu2-112 leu2-3 ura3 his3 trp1*, was transformed with the

Abbreviations: HSE, heat shock element; HSR, heat shock response; HSTF, heat shock transcription factor.

plasmid GA1659-HSE1 (a kind gift from Dr P. Sorger) by spheroplast transformation (Becker & Guarente, 1991) and the resulting strain (DBY747-HSE1) was used in this study.

GA1659-HSE1 contains a HSE sequence upstream of a *lacZ*-cytochrome *c*₁ gene fusion construct. It also carries a wild-type copy of the *ura3* gene. β -Galactosidase is not expressed at detectable levels in exponentially growing DBY747-HSE1 under normal growth conditions.

Culture conditions. DBY747-HSE1 was grown in minimal medium (w/v: 0.67% yeast nitrogen base, without amino acids, 2.0% glucose) supplemented with histidine (20 mg l⁻¹), leucine (30 mg l⁻¹) and tryptophan (20 mg l⁻¹). Cultures were grown overnight at 25 °C in a shaking waterbath to mid-exponential phase ($2-3 \times 10^6$ cells ml⁻¹).

Stress procedure. Aliquots (20 ml) of exponentially growing cells were placed in 250 ml conical flasks and subjected to one of the following treatments: no addition; 6% (v/v) methanol added; 2.6% (v/v) ethanol added; 1.5% (v/v) *n*-propanol added; 0.5% *n*-butanol added; 0.3% (v/v) *n*-pentanol added. The mouth and neck of each flask was covered with a sheet of clingfilm and secured with masking tape to prevent evaporation of alcohol. The flasks were placed in shaking waterbaths at the appropriate temperature for 1 h.

Cells were harvested by spinning at 3000 r.p.m. for 3 min in an MSE bench-top centrifuge. The pellets were resuspended in 1 ml ice-cold Z-buffer (Miller, 1972) (0.05 M sodium phosphate pH 7.0, 0.01 M potassium chloride, 1 mM magnesium sulphate, 0.05 M β -mercaptoethanol) and transferred to 1.5 ml Eppendorf tubes. These tubes were spun at 13000 r.p.m. in an MSE microfuge for 1.5 min and the supernatants discarded. Glass beads (0.4 mm; Sigma) equal in volume to the cell pellet, were added to the tubes and 20 μ l Z-buffer was also added. The cells were disrupted by whirlimixing the samples for 30 s, on twelve occasions, and cooling them on ice for 1 min between each burst of treatment. A further 80 μ l Z-buffer was added to each sample, whirlimixed briefly and spun for 5 s at 13000 r.p.m. in an MSE microfuge. The β -galactosidase activity and protein concentration in the supernatants were measured.

β -Galactosidase assay. *o*-Nitrophenol β -D-galactopyranoside (ONPG) hydrolysis was used to monitor β -galactosidase activity: 20 μ l supernatant was added to a 1 ml solution of 0.2 ml Z-buffer, 0.2 ml ONPG (2 mg ml⁻¹ in water), 0.6 ml distilled water and incubated for 30 min at 37 °C. Absorbance readings were taken at 430 nm. The β -galactosidase activity was expressed in units defined as the change in absorption at 430 nm in 30 min (mg protein)⁻¹. Bio-Rad protein reagent was used according to the manufacturer's instructions to estimate the total protein content in the supernatants.

RESULTS

The presence of alcohol lowers the temperature of maximal HSR

In preliminary experiments (data not shown), the concentration of alcohol that produced a maximal heat shock response profile was established for each of five alcohols. These concentrations were then used in a comparative study on the effect of carbon-chain length on the heat shock response. The results of this study are presented in Fig. 1. In the interests of clarity the data are presented as two graphs.

In the absence of alcohol, exponentially growing cells showed low-level induction of β -galactosidase activity at

33 °C. This gradually increased until it peaked at 40 °C before dropping rapidly, due to cell death, as the temperature increased to 43 °C.

In the presence of alcohol, exponentially growing cells had a 3–5-fold higher level of β -galactosidase induction at 33 °C than that found in untreated control cells. The level of induction gradually increased over the next 4 °C, peaking at 37 °C, before dropping rapidly, due to cell death, as the temperature increased further. Alcohol-treated cells showed a similar pattern of response, irrespective of carbon-chain length, with the maximum heat shock response occurring at 37 °C in each case.

These results are consistent with alcohol and temperature having synergistic effects on the cell's HSR. They suggest that alcohols mediate a reduction in both the minimum and maximum temperatures required for the HSR in *S. cerevisiae*.

The concentration of alcohol required to lower the threshold of maximum HSR decreases with increasing carbon-chain length

A semi-log plot of the alcohol concentration required to produce a maximal HSR versus the number of carbons in the stressing alcohol, is presented in Fig. 2. It reveals a straight-line relationship between these two variables.

Fig. 1. Alcohols lower the temperature of maximal HSR. *S. cerevisiae* DBY747-HSE1 was grown overnight at 25 °C and subjected to one of the following treatments. (a) No addition ($\square$); 1.875 M methanol added ($\blacksquare$); 0.565 M ethanol added ($\circ$). (b) No addition ($\square$); 0.233 M *n*-propanol added ($\bullet$); 0.068 M *n*-butanol added ($\triangle$); 0.034 M *n*-pentanol added ($\blacktriangle$). Samples were incubated for 1 h at the indicated temperatures. (It was technically impossible to process replicate samples for this simultaneous comparisons of six treatments at twelve different temperatures but repeated experiments comparing individual alcohols and the control also revealed this shift.)

Fig. 2. The concentration of alcohol required to lower the threshold of maximal HSR decreases with increasing carbon-chain length. The logarithm of the alcohol concentrations required to induce the maximum HSR was plotted against the number of carbons in the alcohols.

If membrane perturbation is involved in the HSR, agents that interact with the cell membrane should affect the response. The lipid:water partition coefficient of alcohols increases with increasing carbon-chain length (Katz & Diamond, 1974). That a proportionally lower concentration of alcohol elicited the same shift in the maximum HSR threshold as the carbon-chain length increased, supports the hypothesis that the membrane plays a role in modulating the cell's response to an increase in temperature.

DISCUSSION

A large number of studies have been conducted on the physiological responses of microbial cells to the presence of alcohols (for reviews see O'Neal Ingram & Buttke, 1984; Jones, 1989). Leão & van Uden (1982) demonstrated that the concentration of alcohol required to kill yeast cells decreased as the carbon-chain length of the alcohol increased. Alcohol has also been shown to induce heat-shock-inducible genes in yeast in the absence of elevated temperatures (Plesset *et al.*, 1982) and indeed Kirk & Piper (1991) used high concentrations of methanol to induce high level expression from a heat-shock-inducible expression vector in *S. cerevisiae* at its normal growth temperature of 25 °C.

This is the first paper to present a systematic examination of the effect that alcohol concentration and carbon-chain length have on the ability of yeast cells to mount a stress response in the face of elevated temperatures. It demonstrates that alcohol can lower the temperature required for the maximum induction of a heat-shock-inducible promoter in the yeast *S. cerevisiae* by 3 °C (Fig. 1) and that the concentration needed to achieve this decreases as alcohol hydrophobicity increases (Fig. 2). Similar correlations have been found between members of this alcohol series and their effect on a range of membrane functions, including spin label mobility in a lipid bilayer (Paterson

et al., 1972), the ionic permeability of a synthetic lipid membrane (Gutknecht & Tosteson, 1970) and the structural stability of erythrocyte membranes (Seeman, 1966). Furthermore, in the case of the spin label and ionic permeability papers, it was clearly shown that these alcohols had a profound effect on the structural organization of phospholipids.

The hydrophobic nature of the longer-chain alcohols ensures that they preferentially partition into the lipid bilayer of the cell, and the correlation depicted in Fig. 2 is typical of the type of result that implicates a hydrophobic action site in the physiological system under investigation (Thevelein *et al.*, 1979). Our results therefore suggest that the lipid membrane plays a critical role in the physiology of the cell's heat shock response. The yeast cell membrane is much more complex than the synthetic bilayers used in the spin label mobility and ionic permeability studies mentioned above and the membrane-alcohol interaction is therefore difficult to interpret unequivocally. This study does not seek to elucidate the precise molecular target involved but rather to lend support to a model for the heat shock response in which environmental stress is transduced into a cellular signal at the level of the cell membrane.

Yeast cell membranes contain a 100 kDa ATPase protein that maintains a transmembrane electrochemical gradient by pumping H⁺ ions out of the cells (Serrano 1988; Ulaszewski *et al.*, 1983). The activity of this H⁺-ATPase influences the resistance of yeast cells to a variety of stresses (including heat shock) (Panaretou & Piper, 1990; Coote *et al.*, 1991), and Coote *et al.* (1991) have demonstrated that heat stress causes a partial dissipation of the H⁺ gradient that exists across the cell membrane. It is possible that the presence of alcohol in the medium allows this dissipation to occur at a temperature lower than that at which it normally occurs, with the subsequent activation of the stress response genes. Future experiments should reveal if this is indeed the case.

ACKNOWLEDGEMENTS

B. P. G. Curran gratefully acknowledges support from the SGM research fund. We thank Dr V. Bugeja for helpful discussions, and J. Smith and G. Cohen for preparing the manuscript.

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Received 8 December 1993; revised 15 March 1994; accepted 12 April 1994.