Arsenic Oxide-Induced Thermotolerance in *Saccharomyces cerevisiae*

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The growth response of *Saccharomyces cerevisiae* to arsenite and arsenate and the relationship between the enhancement of heat shock protein (hsp) synthesis caused by these arsenic oxides and thermotolerance are reported. Arsenite and arsenate transiently inhibited cell growth and overall protein synthesis; arsenate enhanced the synthesis of the 42-, 74-, 84-, and 100-kilodalton hsps, whereas arsenite enhanced synthesis of only the 74-kilodalton hsp. The induction of these hsps reached a maximum 45 min following metal oxide treatment and then declined. A delayed thermotolerance peaked 4 h after metal oxide addition, at which time cell growth and protein synthesis were recovering. These data show that the arsenate- and arsenite-induced thermotolerance in *S. cerevisiae* cells does not appear to be causally related to either hsp synthesis or cell cycle arrest.

Exposure of organisms to a variety of environmental stressors, including the metal oxides arsenite, AsO_2^- (6, 14), and arsenate, AsO_4^{3-} (5), elicits a cellular stress response (10, 14). In some cases, this response confers cellular tolerance to subsequent stress. For example, heat shock treatment of *Saccharomyces cerevisiae* at 37°C confers cellular tolerance (thermotolerance) to a 50°C treatment which would otherwise kill more than 99% of the cells within 10 min (2, 8). Exposure to ethanol can also confer thermotolerance (12). Neither arsenic oxide has been shown to induce such thermotolerance, however. Cross-resistance is of interest with respect to the mechanism associated with the stress response and its role in conferring tolerance.

Heat shock induces the synthesis of a group of polypeptides known as heat shock proteins (hsps) in yeasts (8) and other organisms (10, 14). The induction by heat shock of hsps has been linked to cellular thermotolerance, although the contributions of individual hsps to this adaptation remain open to question (2). Ethanol and arsenite induce some of the hsps (6, 12, 15). However, whether or not the induction of these stress-induced polypeptides is causally related to any induced thermotolerance remains unclear. Arsenite (0.75 mM) has been reported to cause a slight increase in hsp synthesis in *S. cerevisiae* (70-, 85-, and 98-kilodalton [kDa] hsps; 15). Whether arsenate also induces hsps in yeast cells and whether any thermotolerance can be induced by either arsenic oxide have not been examined previously.

This study attempts to delineate the stress responses induced by arsenite and arsenate in *S. cerevisiae*. The data suggest that both compounds transiently induce some of the hsps as well as a delayed thermotolerance. However, the asynchrony of the two events suggests that the induction of the hsps per se is not sufficient for cell adaptation to thermal stress.

Strain, conditions of growth and labeling, and thermotolerance. The strain used was AS2-2A *MATa trp1 MAL* (13). The medium used for growth and pulse-labeling experiments contained yeast-nitrogen base (6.7 g/liter) buffered with 100 mM Tris-succinate (pH 6.0) and supplemented with 1-tryptophan (280 mg/liter) and 2% dextrose (wt/vol). Yeast extract-peptone-dextrose plates were used to screen for sur-

vival in thermotolerance experiments. Culture growth (Fig. 1) was monitored by measuring turbidity with a Klett colorimeter (100 Klett units, 1.7×10^7 cells per ml). To measure incorporation of [^{35}S]methionine into trichloroacetic acid-precipitable protein (Fig. 2), cells in exponential growth phase (30 to 40 Klett units) were untreated or were treated with arsenite or arsenate. At various times, samples were removed, washed, suspended in fresh media (no metal oxide present), and pulse-labeled with [^{35}S]methionine for 30 min before being quenched with trichloroacetic acid. To determine the effect of arsenic oxides and heat shock on protein synthesis (Fig. 3), exponentially growing cells (74 Klett units) were divided into seven 1-ml aliquots. Five aliquots were incubated with metal oxides (see legend to Fig. 3 for concentrations) for 15 min at 30°C and then labeled with [^{35}S]methionine (20 μCi per sample) for 15 min. The control aliquot was incubated also for 15 min with no addition prior to labeling with [^{35}S]methionine. The final aliquot was labeled following heat shock (15-min incubation at 39°C). After labeling, cell extracts were prepared and analyzed by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography. Enhancement of synthesis of polypeptides was determined by densitometric analysis of the autoradiographs with an LKB Ultrascan XL laser densitometer. To evaluate thermotolerance, metal oxides were added to cultures which were growing exponentially (40 to 100 Klett units). Survival of the pretreated cells was determined before and after a 10-min 50°C treatment.

Effect of metal oxides on growth and protein synthesis. Representative growth curves for strain AS2-2A in the presence of three concentrations of arsenate and arsenite are shown in Fig. 1. Both metal oxides inhibited growth initially. However, cells were able to recover from this inhibition at all concentrations of arsenate and at 0.1 and 1 mM arsenite because the 24-h culture density achieved in the continuous presence of either of the species was essentially at control levels. The transient effects of the two arsenic oxides on growth were similar to that of a related metal oxide, vanadate (VO_4^{3-}). Vanadate at 0.5 mM causes a 6-h growth inhibition (16).

The inhibition by either metal oxide of incorporation of [^{35}S]methionine into the trichloroacetic acid-precipitable fraction in whole cells is illustrated in Fig. 2. These data

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FIG. 1. Growth response of *S. cerevisiae* to arsenic oxides. Sodium arsenate (A) and arsenite (B) were added to cells in exponential growth phase, as indicated by the arrows ($t = 0$). The concentrations of metal oxide added were 0 ($\square$), 0.1 ($\times$), 1.0 ($\blacksquare$), and 5.0 ($\blacktriangle$) mM.

show that this inhibition was transient also, with recovery at 4 to 6 h after arsenic oxide addition. This result suggests that inhibition of protein synthesis was responsible in part for the transient growth arrest. The steady decline in [35 S]methionine incorporation as cultures passed out of exponential growth (cf. Fig. 1 and 2) has been reported previously (1). This occurred in the control cultures after 2 h and in the treated cultures after 6 h, at which times the Klett values were >100 .

Protein synthesis patterns in metal oxide-treated cells. The pattern of protein synthesis in cells treated with the arsenic oxides and heat shock was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography (Fig. 3). The bars separating the heat shock and arsenate lanes indicate those polypeptides whose synthesis was enhanced as a result of heat shock. Arsenate consistently enhanced the synthesis of the four largest of these polypeptides, i.e., of the 42-, 74-, 84-, and 100-kDa proteins. The relative increase in species of lower molecular masses also seen in this autoradiograph was variable. Arsenite enhanced the synthesis of only the 74-kDa species in all experiments ($n = 6$); the 84- and 100-kDa polypeptides exhibited a variable induction. Vanadate (up to 5 mM) did not induce the synthesis of any of the polypeptides detected in the gels (data not shown). Note that the arsenic oxides and heat shock inhibited the synthesis of some of the polypeptides resolvable in these gels.

FIG. 2. Effect of arsenic oxides on incorporation of [35 S]methionine. Cells in exponential growth phase (30 to 40 Klett units, $t = 0$ [cf. Fig. 1]) were treated with 1 mM arsenite ($\blacksquare$) or 5 mM arsenate ($\blacktriangle$) and pulse-labeled with [35 S]methionine at the times indicated. Values represent the label in the trichloroacetic acid-insoluble cell fraction. Control is indicated ($\square$).

To correlate the expression of these metal oxide-induced proteins with the inhibition and recovery phases of culture growth characterized above, extracts from cultures in these various phases were screened by electrophoresis. These proteins returned to control levels by 7 h after metal oxide addition, indicating that their synthesis was diminished in the recovery phase of culture growth (data not shown). This shutoff in synthesis was true of the hsp's as well, a phenomenon which has been well documented in yeasts (9).

Arsenic oxide-induced thermotolerance. To determine if arsenic oxides also induced thermotolerance, two experimental designs were employed. Immediate thermotolerance was assessed by treating cells with metal oxide at 30°C for 1 h prior to a 50°C heat treatment. Survival of these cultures was then determined. While a preincubation at 39°C afforded

FIG. 3. Differential effects of arsenic oxides and heat shock on protein synthesis. Soluble protein extracts of [35 S]methionine-labeled cells were analyzed by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then autoradiographed. This film is representative of six separate experiments. The bars on the film adjacent to lanes HS (heat shock) and Aa (arsenate) indicate polypeptides enhanced by heat shock. The molecular masses of these proteins were 100, 84, 74, 42, 37.5, 32, 27, 24, and 22 kDa. Molecular mass (MW) standards (in kilodaltons) are at left. Sample treatments were as follows: lane C, control (no treatment); lane HS, heat shock; lanes Aa, arsenate at 0.6 and 1.2 mM; and lanes Ai, arsenite at 0.1, 0.25, and 0.5 mM.

TABLE 1. Immediate thermotolerance and thermal or metal oxide pretreatment^a

Pretreatment	Thermal treatment (50°C, 10 min)	Viable cells/ml (10 ⁻⁷) ^b	% Survival ^c
None	—	6.58 ± 1.80	
	+	0.36 ± 0.06	5.5
39°C, 30 min	—	5.84 ± 0.60	
	+	4.58 ± 0.24	78.4
Arsenate (0.1 mM, 1 h)	—	5.18 ± 0.78	
	+	0.08 ± 0.02	1.5
Arsenite (0.1 mM, 1 h)	—	5.34 ± 0.98	
	+	0.02 ± 0.00	0.4
Vanadate, (0.5 mM, 1 h)	—	6.62 ± 0.52	
	+	0.14 ± 0.02	2.1

^a Cell survival following pretreatment alone (—) or pretreatment and exposure to 50°C (+) was determined by plating on yeast extract-peptone-dextrose. Cells were diluted to yield 50 to 200 colonies per plate and incubated for 48 h at 30°C.

^b Calculated on the assumption that each colony was derived from a single cell.

^c For each pretreatment, the number of viable cells per milliliter in the control sample (no 50°C treatment) was taken as 100% survival.

thermotolerance to an immediate 50°C heat treatment, none of the metal oxide treatments induced thermotolerance (Table 1). Note that cells pretreated with arsenate or arsenite at 0.1 mM were still inducible to thermotolerance in a 39°C incubation prior to the 50°C treatment (data not shown).

Delayed thermotolerance was evaluated by incubating cultures with various metal oxide concentrations at 30°C for up to 8 h prior to the 50°C heat treatment and by subsequent evaluation of cell survival. Induction to thermotolerance 4 h after treatment with arsenite was concentration dependent (Fig. 4). Arsenate (0.5 mM) had a similar although quantitatively less pronounced effect (25 to 30% survival at 4 h). In comparison, vanadate at both 0.5 mM (Fig. 4) and 1 mM (data not shown) did not induce thermotolerance. Note that at the metal oxide concentrations described in Fig. 4, arsenite, arsenate, and vanadate arrested growth initially, an

FIG. 4. Concentration dependence of delayed thermotolerance caused by arsenite. Cultures in exponential growth (30 Klett units) were incubated with metal oxide at 30°C for the times indicated prior to treatment at 50°C, dilution, and plating. The values are given as percent survival (cf. Table 1). The concentrations of arsenite used were 0.1 (□), 0.25 (■), and 0.75 (▲) mM. A vanadate control (0.5 mM) is also shown (△). The value X was obtained at 0.5 mM arsenite in a separate experiment. The bar indicates ± the standard deviation ($n = 5$) characteristic of all the data points.

effect which was reversed within 6 to 8 h despite the continuous presence of the metal oxide (Fig. 1; 16).

Relationship between growth arrest, hsp synthesis, and induced thermotolerance. The enhancement of hsp synthesis reached a maximum 45 min following metal oxide addition, returning to control levels in 7 h. A similar temporal pattern was observed in heat-shock yeast cells (9; data not shown). While induction by heat shock of hsps in yeast cells correlates with immediate thermotolerance (8; data not shown), arsenate- and arsenite-induced thermotolerance became significantly only 4 h after metal oxide treatment. By this time, protein synthesis had recovered to control levels (in comparison with levels in control culture in an equivalent state of exponential growth) and the enhancement of stress protein synthesis was markedly diminished. Moreover, arsenate induced more hsps than did arsenite, yet it was less effective at conferring thermotolerance. These results suggest that the observed delayed thermotolerance was not due simply to the presence of one or more hsps induced by either of the arsenic oxides.

The degree of thermotolerance was proportional to the concentration of arsenic oxide added. This result suggests that the delayed thermotolerance was an arsenic oxide-specific event. However, the thermotolerance caused by the arsenic oxides could be related simply to their inhibition of cell growth. That is, *S. cerevisiae* responds to an environmental stress such as nutritional starvation, for example, by progression to a resting state, G_0 (2–4, 7). In this state, the higher-molecular-weight hsps are preferentially synthesized and the cells are more resistant to heat shock (3). Arsenite and arsenate could have diverted cells to this arrested, G_0 state in which they exhibited some thermotolerance. Our data are not consistent with this hypothesis, however. First, vanadate caused the same growth inhibition-recovery behavior (16) but did not induce thermotolerance or hsp synthesis (Fig. 4 and data not shown). Second, the thermotolerance caused by arsenic oxides, although delayed, peaked 4 h after arsenic oxide addition, at which time recovery of growth was already apparent (Fig. 2). These facts indicate that the thermotolerance observed here was not confined to a specific arrested state in the cell cycle.

The mechanism by which these metal oxides alter cell growth is not known. Vanadate and arsenate are both phosphate analogs; both species appear to interfere with cellular phosphorylation reactions (11). In fact, a vanadate-resistant mutant derived from AS2-2A (17) was more resistant to arsenite and arsenate than was AS2-2A itself, also suggesting some common feature in the metabolism of these metal oxides (data not shown). However, the arsenic and vanadium oxides did have quite different effects on the pattern of protein synthesis and cellular thermotolerance. Thus, the sensitivity of yeasts to metal oxides may be associated with distinguishable metabolic fates for these toxic species.

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