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Heat-Induced Expression and Chemically Induced Expression of the *Escherichia coli* Stress Protein HtpG Are Affected by the Growth Environment

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Differences in expression of the *Escherichia coli* stress protein HtpG were found following exposure of exponentially growing cells to heat or chemical shock when cells were grown under different environmental conditions. With an *htpG::lacZ* reporter system, *htpG* expression increased in cells grown in a complex medium (Luria-Bertani [LB] broth) following a temperature shock at 45°C. In contrast, no HtpG overexpression was detected in cells grown in a glucose minimal medium, despite a decrease in the growth rate. Similarly, in pyruvate-grown cells there was no heat shock induction of HtpG expression, eliminating the possibility that repression of HtpG in glucose-grown *E. coli* was due to catabolite repression. When 5 mM phenol was used as a chemical stress agent for cells growing in LB broth, expression of HtpG increased. However, when LB-grown cells were subjected to stress with 10 mM phenol and when both 5 and 10 mM phenol were added to glucose-grown cultures, repression of *htpG* expression was observed. 2-Chlorophenol stress resulted in overexpression of HtpG when cells were grown in complex medium but repression of HtpG synthesis when cells were grown in glucose. No induction of *htpG* expression was seen with 2,4-dichlorophenol in cells grown with either complex medium or glucose. The results suggest that, when a large pool of amino acids and proteins is available, as in complex medium, a much stronger stress response is observed. In contrast, when cells are grown in a simple glucose mineral medium, *htpG* expression either is unaffected or is even repressed by imposition of a stress condition. The results demonstrate the importance of considering differences in growth environment in order to better understand the nature of the response to an imposed stress condition.

Since the original description of the heat shock response (44), a wide range of adverse environmental conditions have been found to induce expression of stress proteins. The function of these induced proteins is to protect the cell against the harmful effects of altered environmental conditions. Many of the induced proteins facilitate the adaptation of metabolism to growth under the altered conditions or enable the cell to adapt in order to enhance survival mechanisms (28, 52). The most intensively investigated stress condition is that of heat shock, and among the bacteria, the best-characterized response is that for *Escherichia coli* (30). Among the *E. coli* heat shock proteins (HSPs) are some which function as molecular chaperones or have functions associated with DNA replication, cell division, and maintenance of active protein conformation (7, 21). Other stress conditions have also been shown to result in induction of specific groups of proteins. Such stimulons include those induced due to nutrient starvation (13, 27, 33, 34), nutrient exhaustion (12, 31, 35, 39, 52), heavy metal stress (5, 10, 38, 47), and phage shock (50, 51), as well as those induced following exposure to a range of organic solvents (5, 29, 32, 47). Many of these stimulons contain proteins which overlap especially with proteins belonging to the heat shock stimulon. The fact that specific patterns of proteins are expressed for a particular stress has led to the development of the use of stress proteins to monitor environmental samples for the presence of particular pollutants (4, 16, 47, 49).

In *E. coli*, regulation of the HSPs involves an alternative sigma factor, σ^{32}, which when bound to the DNA polymerase holoenzyme recognizes the promoter sequence of the HSPs. Under conditions where accumulation of misfolded or dissociated proteins occurs in the cytoplasm, the amount, stability, and activity of σ^{32} increase (6, 15, 42, 54). In addition, another sigma factor, σ^{E}, which recognizes the *E. coli* σ^{32} gene and several other heat shock promoters, is generated in response to signals of stress, such as unfolded proteins, in the periplasm (36, 40).

One of the originally described *E. coli* HSPs is the protein HtpG (C62.5). This protein comprises a large fraction (0.36%) of all proteins in *E. coli* growing at 37°C (20). HtpG possesses a noticeable sequence homology among prokaryotes and eukaryotes (2), with 41% identity in amino acids between the *E. coli* HtpG and its analog in *Drosophila melanogaster. htpG* deletion mutants in *E. coli* grow normally at 37°C but require the protein for growth above 46°C (3, 43). The protein is a dimeric phosphoprotein which has been shown to act as a suppressor of the *secY24* mutation (45) and to suppress growth retardation of an *ftsH* mutant (41). In *E. coli*, in addition to heat, *htpG* expression has been shown to be induced by treatment with a variety of chemicals including ethanol, cadmium chloride, and 2,4-dinitrophenol (11, 46). In yeasts and humans, the analog of HtpG is the Hsp90 protein. This is also a highly abundant molecular chaperone which serves to protect proteins from denaturation on temperature upshifts. The protein is thermally stable up to 50°C but is very sensitive to the levels of divalent cations (22).

Since a stress or a shock involves a change from one envi-

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ronmental condition to another, the nature of the original condition plays a major role in defining the response which is required. Nevertheless, most of the work that has been carried out on stress has focused more on the nature of the stress than on the environment within which the stress is applied. With HtpG as an HSP marker, it has already been shown elsewhere that, in continuous culture, the growth environment has a strong influence on the nature and extent of stress gene expression (19). The work presented here extends this approach and examines how the nature of the growth environment affects the stress response to both a temperature-induced stress and a chemically induced stress. The results show that when a large pool of amino acids and proteins is available, as in a complex medium, a much stronger stress response is observed. In contrast, when cells are growing in a simple glucose mineral medium *htpG* expression either is unaffected by or is even repressed by imposing a stress condition.

MATERIALS AND METHODS

Bacterial strains. *E. coli* JB23 (MC1655 F^- *lacZ*::Tn*10 zba315*::*kan* Δ*htpG1*::*lacZ*) and JB22 (MC1655 F^- *lacZ*::Tn*10 zba315*::*kan*) were kindly provided by E. A. Craig. JB23 contains a chromosomal substitution deletion mutation where the coding sequence of the *htpG* gene has been replaced by the coding sequence of the *lacZ* gene in a *lacZ* mutant, resulting in an in-frame fusion between the codons for amino acid 15 of HtpG and amino acid 8 of β-galactosidase. JB22 is identical, except that the *htpG* gene is intact. A detailed description of the strains and their construction is given by Bardwell and Craig (3). Control experiments comparing growth of *E. coli* JB22 and that of *E. coli* JB23 showed that there was no difference in the growth rates of the two strains, indicating that the *htpG* deletion had no effects on normal growth (data not shown). Similarly, it has been shown previously that growth of the mutant strain was indistinguishable from that of the wild type at temperatures below 46°C (43).

Growth conditions. Bacteria were grown in batch culture in a defined mineral salts medium (8) modified by substituting 29.3 mg of Na_2-EDTA/liter for citrate. The medium was supplemented with 0.2% of the respective carbon source (glucose, glycerol, and pyruvate). For experiments carried out with complex medium, the Luria-Bertani (LB) medium containing (per liter) 3 g of K_2HPO_4, 1 g of KH_2PO_4, 10 g of tryptone, 5 g of yeast extract, and 5 g of NaCl was used. For routine growth, kanamycin (100 mg/liter) was added to the culture. Stress experiments were carried out in the absence of kanamycin. Growth was monitored by measuring the absorption of samples at 546 nm in a Uvikon spectrophotometer (Kontron, Zurich, Switzerland).

Stress experiments. Parallel cultures of *E. coli* JB23 were inoculated in Erlenmeyer flasks from cultures transferred twice in either modified Evans medium or LB medium. Cultures were grown at 37°C until an optical density at 546 nm (OD_{546}) of 0.4 was attained. For heat shock experiments, whole flasks were transferred to a second water bath operating at 45°C and either maintained at this temperature for the duration of the experiment or transferred back to 37°C after 15 min. For chemical stress experiments, appropriate quantities of phenol or 2-chlorophenol were added directly to the cultures once an OD_{546} of 0.4 had been attained. 2,4-Dichlorophenol was added together with ethanol as the solvent. Samples of the culture were withdrawn periodically for determination of β-galactosidase and were immediately frozen in liquid nitrogen and stored at −18°C.

β-Galactosidase assay. Samples were thawed on ice at 4°C. Five milliliters of the sample was washed twice by centrifugation for 6 min at 36,000 × *g* in a buffer containing 0.02 M Na_2HPO_4 at pH 7. Three milliliters of the washed-cell suspension was then transferred to a precooled glass tube, and the cells were disrupted by sonification (Branson sonifier 450; Skan) with a duty cycle of 40% and an output control of 2. The container holding the cells was maintained in ice during the sonification period (three times, 2 min each) to ensure that the sample remained cool. The samples were maintained on ice prior to subsequent analysis. β-Galactosidase was assayed according to the method described by Miller (37), modified so that the rate of increase in the absorbance was measured at 420 nm with a Uvikon 860 UV-visible light (UV/VIS) spectrophotometer (Kontron). Relative specific activities are expressed in arbitrary units and defined as enzyme activity per unit of OD_{546}, normalized to the specific activity measured in the control unstressed cultures. Duplicate measurements within an experiment gave less than 10% variation.

Data analysis. Shown in the figures are data from single representative experiments. All experiments were repeated several times to ensure reproducibility of the results. Statistical analysis was performed with the independent *t* test to determine the significance of differences between conditions tested, significance being ascribed at $P > 0.1$.

RESULTS

Heat shock in rich medium. The macroscopic effects of growth perturbations are most easily seen as changes in growth rate. Representative batch growth curves from experiments where *E. coli* JB23 was subjected to either a prolonged or a temporary heat shock are shown in Fig. 1A. In these experiments, *E. coli* JB23 was grown in a rich nutrient medium (LB broth). Analysis of the growth rates following the stress (Table 1) shows that the prolonged shock had a more substantial effect on the growth rate than did the transient heat shock. Batch growth became limited, presumably by oxygen, in the Erlenmeyer shake flasks used during the late exponential phase as indicated by the gradual decline in the growth rate following the extended period of logarithmic growth. Figure 1B shows a good example of the classical heat shock response exemplified here by the expression of the heat shock gene *htpG* measured as β-galactosidase expression from an *htpG* promoter. A transient heat shock resulted in a transient expression of β-galactosidase. This peaked 20 min after the temperature upshift at a level that was 2.27 times that of the control and then decreased. Similarly, the level of β-galactosidase measured in the culture that was maintained at 45°C peaked after 20 min, after which it remained at an elevated level equivalent to 1.95 times that of the untreated control after 90 min.

HtpG expression following heat shock to glucose-grown *E. coli*. When *E. coli* JB23, growing in a mineral medium with glucose as the carbon source, was subjected to the same heat shock conditions, the growth rate decreased to 77% of that of the unstressed control following the transient (15 min) heat shock at 45°C (Fig. 2A). Growth in the culture which was maintained at 45°C slowed rapidly after 20 min to 50% of that of the control, while the culture transferred back to 37°C recovered its growth rate to approach that of the culture maintained at 37°C. Expression from the HtpG HSP promoter remained unaffected by any of the heat treatments despite the effects on the growth rate of the culture (Fig. 2B). Even in the culture that was maintained at 45°C, there was no enhanced expression of β-galactosidase from the *htpG* promoter when cells were subjected to a heat shock while growing in a glucose mineral medium.

Expression of HtpG following heat shock to *E. coli* grown on pyruvate. Since expression of the gene encoding the sigma factor (σ^{32}) required to recognize the heat shock gene promoters is partially affected by catabolite repression (14), it was considered possible that this caused the lack of enhanced HtpG synthesis following a heat shock in *E. coli* JB23 grown in a glucose mineral medium. In order to determine whether this was indeed caused by glucose catabolite repression, the same *E. coli* strain was grown on pyruvate as the sole carbon energy source in a mineral medium and subjected to the same heat shock regimens as described above. Heat stress caused a reduction in the growth rate following a transient (15 min) heat shock at 45°C, where it fell to 0.89 relative to the unstressed growth rate (Table 1). Following a return to 37°C, the culture recovered its pre-heat shock growth rate. When the temperature was maintained at 45°C, the relative growth rate decreased to 0.62 prior to eventually slowing down to approach zero.

In a manner analogous to that of glucose-grown cells, expression from the *htpG* promoter was unaffected by exposure to heat in any of these experiments with pyruvate as carbon source (Table 2). Identical results were also found in heat-shocked cultures of *E. coli* JB23 when glycerol was used as the sole carbon and energy source.

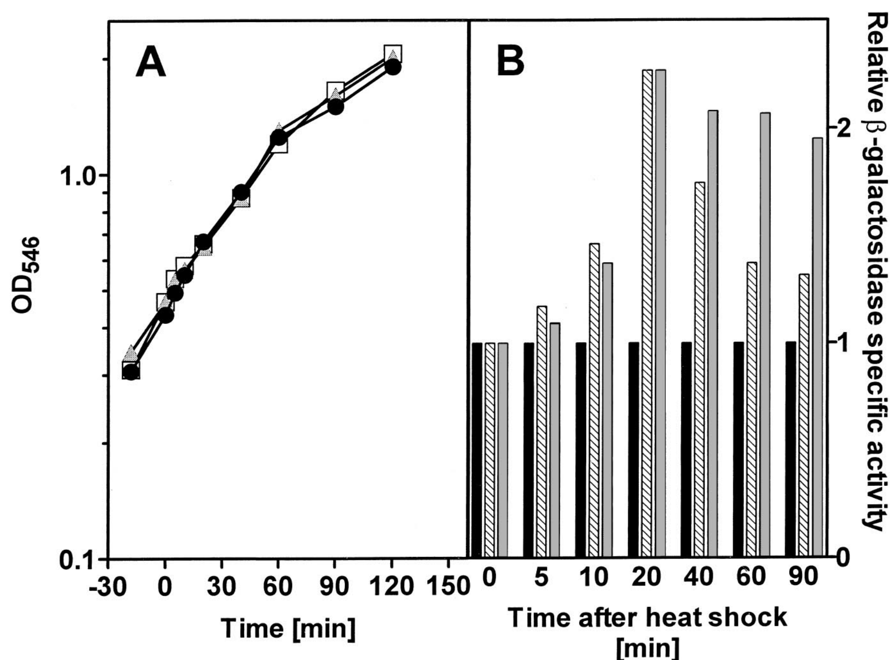

FIG. 1. (A) Growth of *E. coli* JB23 in complex medium exposed to a temperature shock. Growth curves are shown for three cultures. At time zero, one culture (△) was transferred to 45°C and then returned to 37°C after 15 min; a second culture (□) was transferred to 45°C for the remainder of the experiment, while the third culture (●) was maintained at 37°C. (B) Expression of the *htpG* gene was determined as specific β-galactosidase activity of the *htpG-lacZ* fusion protein. ■, control; ▨, transient heat shock; ▦, long-term heat shock.

Effect of chemical stress on expression of HtpG in *E. coli* JB23: phenol. Since there was a strong dependence of growth conditions on the subsequent expression of the *E. coli* HSP HtpG, and it is known that many of the HSPs are expressed under a variety of other stress conditions (1, 5, 23–26, 53), *htpG* expression was examined following chemical stress during growth in rich and in minimal media. Unsubstituted and mono- and dichloro-substituted phenols were used as the chemical stressing agents, and expression of *htpG* was determined based on the level of β-galactosidase protein activity, as with the heat shock experiments. In Fig. 3, the results of addition of various concentrations of phenol to *E. coli* JB23 growing in a rich medium (LB broth) are shown. Following the addition of phenol, there was an immediate decrease in the rate of growth of the bacterial culture, the extent of the decrease being dependent on the concentration of phenol added. When the final concentration of phenol was 5 mM, the growth rate decreased to 81% of that of the untreated control while addition of 10 mM phenol resulted in a reduction in the relative growth rate to 0.53. In both instances, growth was inhibited but not repressed and continued for at least 2 h following the addition of the chemical stressing agent. Expression from the HtpG HSP promoter was 2.4 times that of the unstressed control following exposure to 5 mM phenol. The level of expression increased and reached a maximum at 40 min after exposure. In contrast, exposure to 10 mM phenol resulted in a decrease in the level

TABLE 1. Relative growth rates[a] of *E. coli* JB23 following physical and chemical stress

Stress	Condition(s)	Relative rate in growth medium:			
		LB	Glucose	Pyruvate	Glycerol
Heat shock	37 → 45 → 37°C	0.93	0.77	0.89	ND[b]
	37 → 45°C	0.85	0.50	0.62	ND
Chemical stress:					
Phenol	5 mM	0.81	0.85	ND	0.80
	10 mM	0.53	0.44	ND	0.46
2-Chlorophenol	0.25 mM	0.91[c]	0.99[c]	ND	ND
	0.5 mM	0.86	0.90	ND	ND
	1.0 mM	0.83	0.70	ND	ND
	1.5 mM	0.71	0.51	ND	ND
Ethanol	0.21 mM	0.91[c]	0.99[c]	ND	ND
2,4-Dichlorophenol	0.1 mM	0.71	0.48	ND	ND
	0.2 mM	0.48	0.39	ND	ND

[a] Means of at least two experiments are shown. The results are expressed relative to the growth rate of the unstressed control.
[b] ND, not determined (experiment not carried out).
[c] These values are not significantly different (*t* test) from the control.

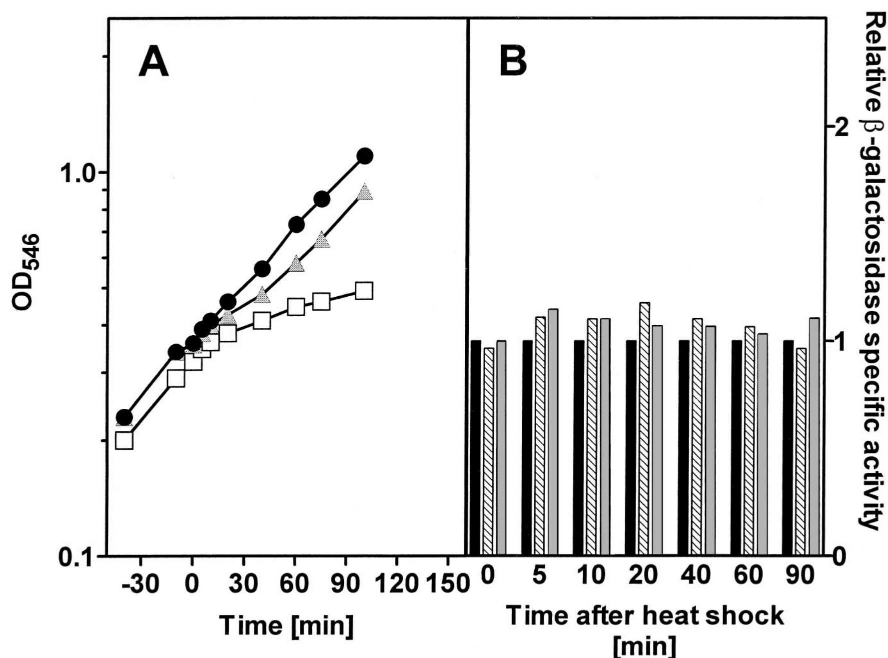

FIG. 2. Growth of and heat shock to *E. coli* JB23 growing in a glucose minimal medium (A) and HtpG expression (B). Symbols are the same as in Fig. 1.

of expression from the *htpG* promoter. The level declined immediately following the stress to a level ca. one-third of that in the unstressed control culture.

Exposure to the same phenol concentration during growth in a glucose mineral medium resulted in a decrease in the relative growth rates of the cultures to 0.85 for 5 mM phenol and to 0.44 for 10 mM phenol (Fig. 4). Expression of β-galactosidase was almost completely repressed during exposure to phenol in glucose-grown *E. coli*, both at 5 mM and at 10 mM phenol, with no recovery seen after 90 min.

As with glucose-grown *E. coli*, addition of phenol to glycerol-grown cultures of *E. coli* JB23 resulted in suppression of β-galactosidase expression both at 5 mM and at 10 mM stress concentrations (Table 2). At the lower concentration, the suppression was temporary, and 90 min after addition, the level of β-galactosidase approached that found in the control culture. Growth rate was suppressed at both concentrations of phenol (0.80 with 5 mM and 0.46 with 10 mM phenol). The growth rates in both phenol-stressed cultures increased about 1 h after addition. This recovery was also seen in the β-galactosidase levels in the culture treated with 5 mM phenol but not with the higher concentration.

Chemical stress with 2-chlorophenol. 2-Chlorophenol also resulted in a reduction in the growth rate of *E. coli* JB23 growing on LB broth (Fig. 5A). The extent of growth rate inhibition was a direct function of the concentration of 2-chlorophenol to which the bacteria were exposed. When cells were stressed with 0.25 mM 2-chlorophenol, growth rate was unaffected, while at higher concentrations, there was a measurable reduction in the growth rate of the bacteria (Table 1). For all concentrations, following the chemical stress with 2-chlorophenol, expression from the *htpG* promoter increased (Fig. 5B).

TABLE 2. Effects of physical and chemical stress on β-galactosidase from the *htpG* promoter

Stress	Condition(s)	Effect in growth medium[a]			
		LB	Glucose	Pyruvate	Glycerol
Heat shock	37 → 45 → 37°C	+	0	0	0
	37 → 45°C	+	0	0	0
Chemical stress:					
Phenol	5 mM	+	−	ND	−
	10 mM	− (0)	−	ND	−
2-Chlorophenol	0.25 mM	+	0 (+)	ND	ND
	0.5 mM	+	− (0)	ND	ND
	1.0 mM	+	−	ND	ND
	1.5 mM	+	−	ND	ND
Ethanol	0.21 mM	0	0	ND	ND
2,4-Dichlorophenol	0.1 mM	0	0	ND	ND
	0.2 mM	0	0	ND	ND

[a] +, increase in expression, −, decrease in expression; 0, no change; ND, not determined (experiment not performed). A symbol in parentheses indicates behavior following prolonged exposure.

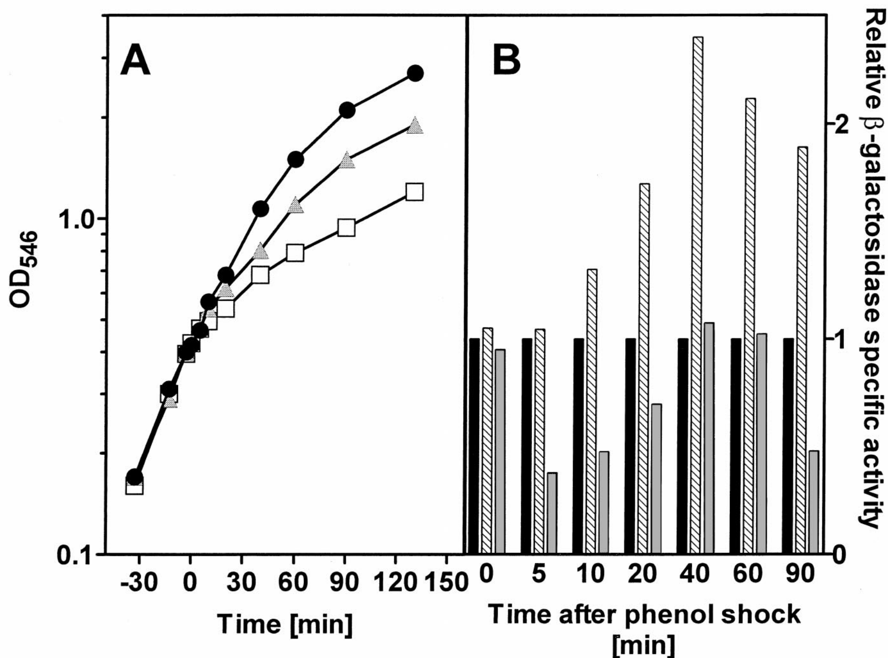

FIG. 3. (A) Growth of *E. coli* JB23 in complex medium exposed to different concentrations of phenol. Cultures were grown to an OD_{546} of 0.4, at which time (0 min) the chemical shock was applied. ●, control; △, 5 mM phenol; □, 10 mM phenol. (B) Expression of the *htpG* gene was determined as specific β-galactosidase activity of the *htpG-lacZ* fusion protein. ■, control; ▨, 0.5 mM phenol; ▦, 10 mM phenol.

This increase was immediate, i.e., within 5 min following the shock. The increase in the level of expression of β-galactosidase was the same and independent of the concentration of 2-chlorophenol used. Even 90 min after the initial shock with 2-chlorophenol, the level of expression from the *htpG* promoter was continuing to increase.

Growth on a glucose mineral medium also resulted in a similar reduction in growth rates during the exponential phase

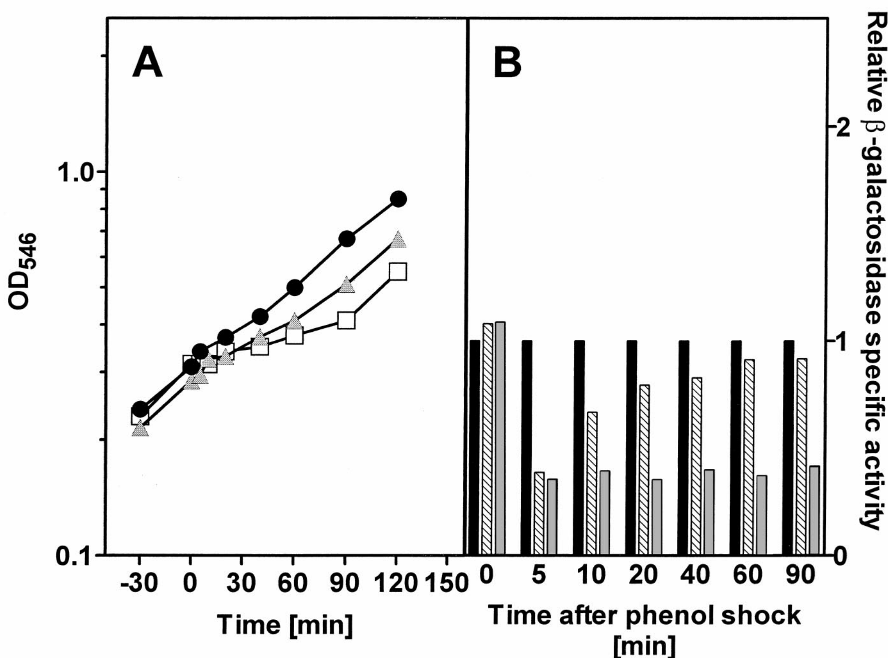

FIG. 4. (A) Growth of *E. coli* JB23 in glucose minimal medium exposed to different concentrations of phenol. Cultures were grown to an OD_{546} of 0.4, at which time (0 min) the chemical shock was applied. ●, control; △, 5 mM phenol; □, 10 mM phenol. (B) Expression of the *htpG* gene was determined as specific β-galactosidase activity of the *htpG-lacZ* fusion protein. ■, control; ▨, 0.5 mM phenol; ▦, 10 mM phenol.

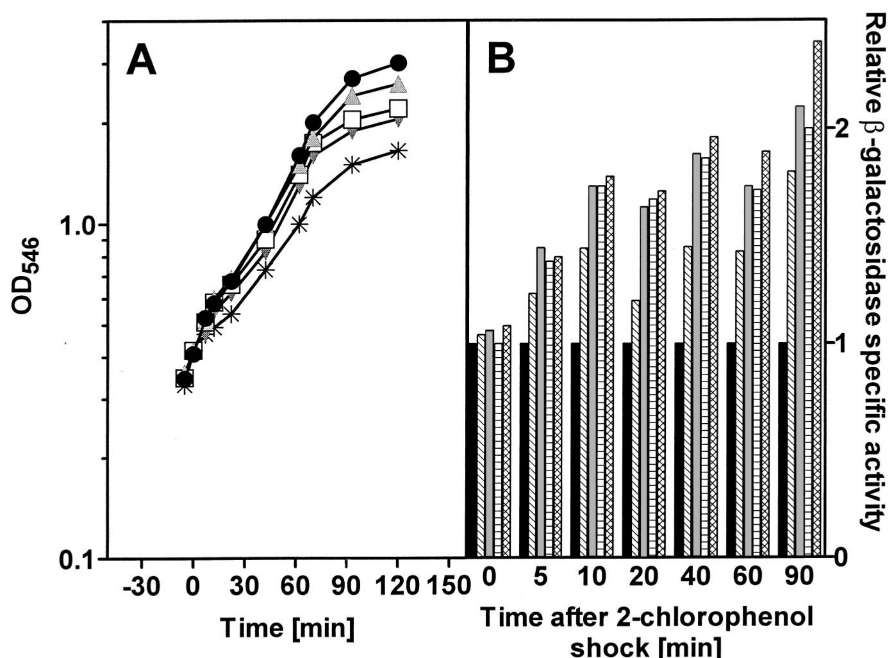

FIG. 5. (A) Growth of *E. coli* JB23 in complex medium exposed to different concentrations of 2-chlorophenol. Cultures were grown to an OD_{546} of 0.4, at which time (0 min) the chemical shock was applied. ●, control; △, 0.25 mM 2-chlorophenol; □, 0.5 mM 2-chlorophenol; ▽, 1 mM 2-chlorophenol; ✳, 1.5 mM 2-chlorophenol. (B) Expression of the *htpG* gene was determined as specific β-galactosidase activity of the *htpG-lacZ* fusion protein. ■, 0 mM 2-chlorophenol; ▧, 0.25 mM 2-chlorophenol; ▦, 0.5 mM 2-chlorophenol; ▤, 1.0 mM 2-chlorophenol; ▩, 1.5 mM 2-chlorophenol.

when 2-chlorophenol was added as a chemical stressing agent (Fig. 6A). The extent of growth rate reduction was also dependent on the concentration of 2-chlorophenol used, with no noticeable change in the relative growth rate with 0.25 mM 2-chlorophenol and an increasing level of repression for the higher concentrations (Table 1). In contrast to the increase in expression from the *htpG* promoter seen for 2-chlorophenol chemical stress in *E. coli* JB23 growing in LB medium, there

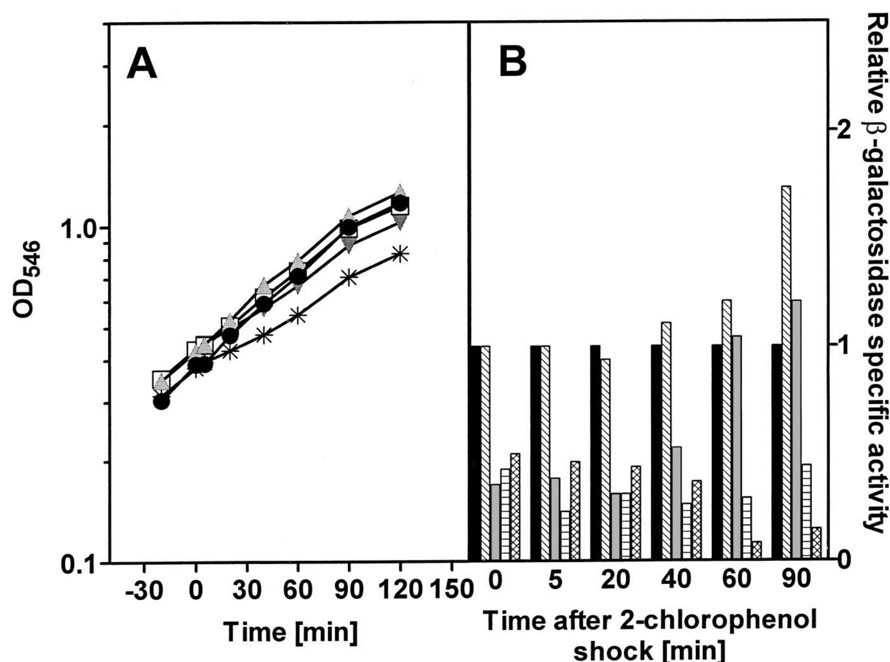

FIG. 6. (A) Growth of *E. coli* JB23 in glucose minimal medium exposed to different concentrations of 2-chlorophenol. Cultures were grown to an OD_{546} of 0.4, at which time (0 min) the chemical shock was applied. ●, control; △, 0.25 mM 2-chlorophenol; □, 0.5 mM 2-chlorophenol; ▽, 1 mM 2-chlorophenol; ✳, 1.5 mM 2-chlorophenol. (B) Expression of the *htpG* gene was determined as specific β-galactosidase activity of the *htpG-lacZ* fusion protein. ■, 0 mM 2-chlorophenol; ▧, 0.25 mM 2-chlorophenol; ▦, 0.5 mM 2-chlorophenol; ▤, 1.0 mM 2-chlorophenol; ▩, 1.5 mM 2-chlorophenol.

was a temporary repression of β-galactosidase expression when the bacteria were subjected to a chemical shock with 2-chlorophenol while they were growing in glucose mineral medium (Fig. 6B). The extent of repression was a function of the concentration of the chemical stressing agent used. For the culture stressed with 0.25 mM 2-chlorophenol, there was no apparent repression of β-galactosidase synthesis. After 90 min following the shock, the level of β-galactosidase actually increased and was overexpressed by a factor of 1.6-fold. Expression from the *htpG* promoter following addition of 2-chlorophenol at higher concentrations resulted in repression of β-galactosidase synthesis. Recovery from this repression occurred with the 0.5 mM 2-chlorophenol-stressed culture in a manner similar to that observed for 0.25 mM but temporally delayed. No recovery occurred with either the 1.0 mM 2-chlorophenol culture or the 1.5 mM culture even at 90 min after the shock.

Expression of HtpG following chemical stress with 2,4-dichlorophenol. Since 2,4-dichlorophenol is very poorly soluble in water, it was added to the cells together with ethanol as a solvent. However, since ethanol itself is known to result in induction of HSPs such as GrpE (36), it is necessary to differentiate the effect of ethanol alone from that of 2,4-dichlorophenol in the presence of ethanol. When cells were grown in complex medium, addition of 2,4-dichlorophenol resulted in a decrease in the growth rate of *E. coli* JB23. The extent of inhibition was concentration dependent. A control culture was also challenged with 0.21 M ethanol. This concentration corresponded to that present together with the 0.2 M 2,4-dichlorophenol. As seen in Table 1, the growth rate reduction was more substantial for cultures grown in the complex medium than for those grown with glucose, while ethanol addition had no significant effect on growth rate reduction in either LB broth or glucose minimal medium. At the higher 2,4-dichlorophenol concentration, the growth rate was measured following addition of 2,4-dichlorophenol, and subsequently the growth rate declined further as the experiment progressed. When cells were grown in complex medium, no difference in the expression of the HtpG reporter, β-galactosidase, was detected with either glucose mineral medium-grown or rich nutrient medium-grown *E. coli* (Table 2).

DISCUSSION

This study demonstrated that the changes in the level of expression of the HSP HtpG following either a heat shock or a chemical shock were dependent on the growth conditions prior to and during the stress. Similar results were found previously for HtpG following a change in temperature from 37 to 42°C in a continuously growing culture of *E. coli* (17–19). For steady-state cultures, the extent of increase in the level of expression of *htpG* was dependent on the nature of the growth medium (19). In this study, we have shown that in response to heat stress (increase from 37 to 45°C), the HSP HtpG is overexpressed when cells are grown in complex medium while its expression remains unchanged during growth of cells on glucose, glycerol, or pyruvate (Table 2). Similarly, it was previously shown that in batch culture the level of HtpG remained unchanged following a shift from 37 to 45°C when cells were grown in glucose minimal medium, while in complex medium HtpG was induced to a ca. 40%-higher level than that at 37°C (17).

HtpG is thought to act as a chaperone in stressed cells, maintaining partially folded proteins in a configuration that facilitates their reactivation by interaction with other chaperones (43). The lack of HtpG in *E. coli* JB23 has no effect on growth at up to 46°C (43), and two-dimensional gel analysis suggests that no induction of synthesis of other proteins occurs to compensate for the absence of HtpG (3). Furthermore, no differences were observed in the sensitivities of the wild type or the *htpG* deletion strain to 256 chemicals, to UV irradiation, or to lambda phage infection (3).

Both *htpG* expression and growth rate were influenced by the pre-stress growth conditions. Temperature shock resulted in a change in the growth rate of *E. coli* JB23 in glucose, glycerol, and pyruvate mineral media but had no noticeable effect on the overall growth rate in complex medium. In contrast, chemical shocks resulted in a reduction in growth rates to approximately the same extent in both minimal medium and complex medium. Complex medium contains a wide range of proteins and amino acids from the hydrolysates of yeast extract and tryptone. Their presence in the growth medium alleviates the need for anabolic synthetic pathways. On the other hand, there is also a larger pool of molecules that can potentially be damaged by exposure to adverse environmental conditions, such as following a temperature shock or as a result of chemical interactions. Thus, changes which are seen in the level of HtpG following a heat shock in complex medium could be a result of multiple sites of damage to the pool of macromolecules. Repair mechanisms have been characterized previously for L-isoaspartyl residues that arise from spontaneous damage to aspartyl or asparagyl residues (48). This pool is not present in cells grown in glucose minimal medium, so that changes in the physical or chemical environmental conditions can be compensated for by changing growth rate rather than by overexpressing particular stress proteins. This suggested model does not exclude the possibility that other heat shock or stress proteins react differently.

One of the hypotheses considered was that catabolite repression might be important in the regulation of expression of the heat shock gene *htpG*. This was considered since induction of *htpG* occurred following a heat shock in complex medium while expression appeared to be unaltered following a heat shock in glucose mineral medium. One of the promoters of *rpoH*, P5, requires activation by the cyclic AMP-catabolite activator protein complex. Control of P5 activity is by catabolite repression and results in a two- to threefold-higher expression of the *rpoH* gene in glucose-free medium (14). However, the results with the glycerol- and pyruvate-grown cultures, which showed a response similar to that of the glucose-grown culture to a heat shock indicated that the differences in expression of HtpG between cells grown in complex medium and those grown in glucose mineral medium did not involve catabolite repression.

Stress proteins are modulators of metabolism, enabling growth to occur unperturbed by changes in environmental conditions or enhancing protection against damage by adverse conditions. These results suggest that studies of stress protein regulation need to be carried out under conditions more akin to real environmental conditions rather than under the ideal conditions often used in many such stress studies. In a separate study, we have also seen a difference in the induction of the *katF* gene, which encodes the σ^S subunit of RNA polymerase and is responsible for induction of the stationary-phase proteins, as a function of the medium in which the cells are grown (data not shown). Furthermore, a recent report on the σ^S-regulated gene *uspB* also described differences in expression in minimal medium and in complex medium (9).

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