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Synergistic Induction of the Heat Shock Response in *Escherichia coli* by Simultaneous Treatment with Chemical Inducers

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*Escherichia coli* strains carrying transcriptional fusions of four or-controlled *E. coli* heat shock promoters to *luxCDABE* or *lux* reporter genes were stressed by chemicals added singly or in pairs. Much more than additive induction resulted from combinations of cadmium chloride, copper sulfate, ethanol, formamide, 4-nitrophenol, and pentachlorophenol.

**Synergistic increases in bioluminescence from a *lon*:*lux* gene fusion.** Two chemical inducers of the heat shock response, pentachlorophenol and ethanol, were added singly and in combination to *E. coli* DPD1006. Figure 1A shows the bioluminescence at 26°C as a function of time. The combination of inducers as well as each individual inducer resulted in transient increases in light output, as previously observed with other strains containing heat shock promoter-*lux* fusions (16, 17). Specific induction units (SIU), representing the increased light output due to the presence of the inducer, normalized to 10^7 cells, were calculated by using the formula SIU = (RLU_c – RLU_u)/10^7 cells, where RLU_c is the bioluminescence of treated cells, RLU_u is the bioluminescence of untreated cells, and the cell density is calculated from the Klett measurement recorded at the beginning of the experiment and the conversion factor of 5.6 × 10^6 cells ml^-1 Klett unit^-1, determined from serial dilution and plating of five independent cultures grown at 25°C to mid-exponential phase. Treatment of strain DPD1006 with pentachlorophenol for 60 min yielded 0.53 SIU; the addition of ethanol yielded 18.0 SIU. The combination yielded 45.1 SIU, which is substantially greater than the sum of the SIU for the individual compounds. Synergistic actions of ethanol and pentachlorophenol were also observed with several other concentrations of pentachlorophenol in the presence of ethanol (Fig. 1B). These more than additive responses suggested synergistic action of pentachlorophenol and ethanol on induction of the heat shock response in *E. coli*. The combination of pentachlorophenol and ethanol does not increase plasmid levels. To investigate if changes in plasmid level were a factor in the bioluminescent response observed, *E. coli* DPD1006 was grown in LB medium at 26°C and treated with 3% (vol/vol) ethanol, 37.5 μg of pentachlorophenol per ml, or a combination of the agents. Light production from 100-μl samples, culture turbidity, and plasmid content were determined 60 min after addition of the chemicals. Similar to the results from microtiter plate induction experiments, the bioluminescence from the cultures treated with either pentachlorophenol (3.83 RLU) or ethanol (21.0 RLU) was greater than that from the untreated cells (0.01 RLU). The combination of pentachlorophenol and ethanol resulted in a level of light production that was significantly greater than additive (40.32 RLU). The plasmid content was determined by densitometry of plasmid DNA isolated by alkaline lysis, linearized with the restriction enzyme EcoRI, separated by agarose gel electrophoresis, and stained with ethidium bromide. Untreated cells were found to have 1.8 × 10^-8 ng of plasmid per cell, while the cells treated with pentachlorophenol and ethanol

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contained $1.6 \times 10^{-6}$ ng of plasmid per cell. Thus, the increases in bioluminescence upon chemical treatment were unlikely due to increases in plasmid levels.

**Synergistic induction from a chromosomal PrpoD$_{hs}$-lacZ fusion.** The effects of combined inducers on a strain with a different reporter of heat shock gene expression was tested with *E. coli* MC4100 lysogenic for λpF13-(PrpoD$_{hs}$-lacZ). Following a 31-min chemical treatment of cells grown in LB medium at 30°C, β-galactosidase activity was assayed with the Galacto-Light chemiluminescent system (Tropix, Inc.) by a modification of the procedure suggested by the vendor. Chloroform (20 µl) was added to the lysis solution (80 µl) for cell permeabilization. After incubation with the chemiluminescent substrate at 26°C, Emerald luminescence enhancer was injected and chemiluminescence was quantitated by using a Dynatech ML3000 luminometer in the enhanced-flash mode. Means of quadruplicate samples are reported. The specific β-galactosidase activity was calculated by normalizing the activity to the cell concentration with the units RLU min$^{-1}$ 10$^{9}$ cells$^{-1}$, where RLU is the light measured, minutes are the duration of the β-galactosidase assay reaction, and cell density is calculated on the basis of the turbidity measurement. The

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**FIG. 1.** Synergistic induction of a *lon*':*lux* fusion by the combination of 37.5 µg of pentachlorophenol (PCP) per ml and 3% (vol/vol) ethanol. (A) Kinetics of light production. (B) SIU at various concentrations of pentachlorophenol. *E. coli* DPD1006 was grown and tested at 26°C in LB medium. SIU were calculated at 60 min after addition of the chemicals to the cells as described in the text.
The synergistic induction of the heat shock response has been observed in mammalian (6, 8, 13–15), fungal (4), and amphibian (7) cells. In four cases, this synergy is at the level of mRNA synthesis (4, 7, 14, 15), suggesting transcriptional control. Our observations with transcriptional fusions are consistent with this phenomenon also occurring at the transcriptional level in bacteria. Synergistic induction thus appears to be another example of the remarkable similarity of the heat shock response in many organisms, which is consistent with its fundamental importance in the response to a variety of stresses.

The synergistic induction of the heat shock response has implications for the application of biosensor strains in environmental stress detection. Environmental samples would likely contain a variety of chemicals resulting in synergistic induction of the heat shock response. The increased bioluminescence represents the increased transcription and toxic effect upon the cell, which result in a decrease of cellular metabolism required for bioluminescence or the function of any of the five lux gene products. Despite this potential underestimation, numerous examples of combinations of chemicals resulting in synergistic induction were observed, suggesting that the nonadditivity is a general phenomenon in E. coli.

Synergistic heat shock protein induction also appears to be conserved in several phyla. There are examples of such synergy in mammalian (6, 8, 13–15), fungal (4), and amphibian (7) cells. In four cases, this synergy is at the level of mRNA synthesis (4, 7, 14, 15), suggesting transcriptional control. Our observations with transcriptional fusions are consistent with this phenomenon also occurring at the transcriptional level in bacteria. Synergistic induction thus appears to be another example of the remarkable similarity of the heat shock response in many organisms, which is consistent with its fundamental importance in the response to a variety of stresses.

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contain many components. The toxicity of such combinations would be difficult to predict. Use of strains containing heat shock promoter-lux fusions would allow detection of subtlethal yet stress-producing environmental conditions. These results suggest that in many cases, multiple toxicants, rather than dampening induction, may enhance it. Furthermore, it may be useful to deliberately add a synergist to expand the range of concentrations and compounds detected.

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