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Unique and Overlapping Pollutant Stress Proteins of *Escherichia coli*

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Exposure of growing batch cultures of *Escherichia coli* to nine different "model micropollutants" (benzene, cadmium chloride, chlorpyrivos, 2,4-dichloroaniline, dioctylphtalate, hexachlorobenzene, pentachlorophenol, trichloroethylene, and tetrapropylbenzosulfonate) led to the induction of 13 to 39 proteins, as analyzed by two-dimensional gel electrophoresis. Some of these proteins overlapped with heat shock and carbon starvation proteins, but at least 50% were unique to a given chemical. The stress protein induction showed a temporal pattern, indicating sequential gene expression. Chemical stress protein synthesis occurred even at concentrations that had no effect on growth. Thus, the synthesis of these proteins can be a sensitive index of stress and the nature of environmental pollution.

Many microbial processes are concerned with their constantly changing external environment (4) to which the organism must respond quickly in order to compete successfully. As a response to harmful environmental conditions, the cell may produce additional proteins, often referred to as stress proteins. Different sets of genes and proteins are induced by different stresses. Well-characterized procaryotic examples include the heat shock response (11), the SOS response (16), oxidative stress (2, 10), starvation response (3, 6, 7, 8), and anaerobiosis (14). Chemicals like $CdCl_2$, H_2O_2, 6-amino-7-chloro-5,8-dioxoquinoline, and ethanol also stimulate the synthesis of stress proteins in *Escherichia coli* and *Salmonella typhimurium*, some of which are unique. Thus, the precise cellular response to a specific chemical stress could be characteristic for that chemical. Studies presented in this article demonstrate that nine common environmental pollutants elicit the synthesis in *E. coli* of a unique set of proteins. This raises the possibility of using protein synthesis pattern in identifying the nature of environmental stress.

MATERIALS AND METHODS

The Stanford *E. coli* K-12 strain was used (6). The culture conditions have been described previously (3). The model pollutants in this study were selected on the basis of their presence in various aquatic environments; they are benzene, cadmium chloride ($CdCl_2$), chlorpyrivos (CPV), 2,4-dichloroaniline (DCA), dioctylphtalate (DOP), hexachlorobenzene (HCB), pentachlorophenol (PCP), trichloroethylene (TCE), and tetrapropylbenzosulfonate (TPBS). Stock solutions of $CdCl_2$ and TPBS were made in water; stock solutions of benzene, CPV, DCA, DOP, PCP, and TCE were made in ethanol (final ethanol concentration, <1% [vol/vol]); and stock solutions of HCB were made in ether (final ether concentration, 0.1%). The final concentrations of the pollutants are specified in Table 1. After 1 h of exposure, ethanol, at the concentration used, did not elicit the synthesis of any stress proteins; ether induced spots 217 and 220 (see Table 3).

The exponential-phase *E. coli* culture was divided into 10-ml aliquots (cell density, ca. 3×10^8 cells per ml), and each aliquot was exposed to one of the pollutants. Doubling times were determined by measuring the optical density at 660 nm. Aliquots exposed to the highly volatile chemicals TCE and benzene were cultured in closed screw-cap bottles with Teflon-silicone membranes to prevent evaporation. TCE was added to a separate tube inside the bottle. Samples were withdrawn with a syringe through the Teflon membrane. Because of possible oxygen depletion and its effects on the induction of stress proteins, a closed control (without chemical addition) was also included.

Cellular polypeptides were labelled and separated by two-dimensional polyacrylamide gel electrophoresis (two-dimensional PAGE) as described previously (3). Samples were pulse-labelled with 10^{-8} M [^{35}S]methionine (110 Ci/nmol) and chased with 10^{-5} M unlabelled methionine. After precipitation, ca. 750,000 counts was loaded on gels for separation of proteins by two-dimensional PAGE (12). Labelled proteins were visualized on XAR-5 film (Eastman Kodak Co.) by autoradiography.

RESULTS

The concentrations of the chosen pollutants produced, in most cases, some growth inhibition (Table 1), as measured after 3 h of exposure. Saturating concentrations of HCB did not produce growth inhibition. For benzene, a concentration that showed no growth inhibition was also analyzed; only minor differences between protein synthesis patterns were noted at the two concentrations. The solvents ethanol and ether did not inhibit growth at 0.1% (vol/vol) or lower; higher ether concentrations were inhibitory, and ethanol at 1% (vol/vol) was sometimes inhibitory (Table 1).

Of the nine chemicals tested, only $CdCl_2$ and the solvent ethanol have been previously shown to elicit stress protein synthesis (14). Before each treatment, a sample was taken from the mid-log phase of the culture for analysis of the unstressed pattern of protein synthesis. Aliquots of the culture were then exposed to the individual pollutants for 15, 30, and 60 min, except for CPV (15, 30, 95, and 180 min) and benzene and TCE (120 and 180 min), and analyzed for protein synthesis. Visual analysis of the autoradiographs

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TABLE 1. Effect of pollutants on *E. coli* growth rate

Treatment	Concn[a]	Doubling time (% of control)
Open control[b]	0	100[c]
Closed control[d]	0	100
Ethanol	0.1% (vol/vol)	100
Ethanol	1.0% (vol/vol)	100
Ether	0.1% (vol/vol)	100
Ether	1.0% (vol/vol)	200
Benzene	2,670 mg/liter	131
$CdCl_2$	80 mg/liter	150
CPV	390 mg/liter	243
DCA	102 mg/liter	120
DOP	0.30 mg/liter	120
HCB	0.01 mg/liter	100
PCP	3 mg/liter	138
TCE	0.009 mg/liter	131
TPBS	1,000 mg/liter	120

[a] Actual concentrations of the chemicals in the culture were not measured, and therefore the values are approximate.
[b] Every experiment included an unexposed control culture to allow for correction of minor differences between different experiments.
[c] Doubling times of the controls varied from 75 to 100 min in different experiments.
[d] The benzene and TCE experiments included an unexposed culture in a closed bottle to allow for correction of differences caused by the different experimental setups.

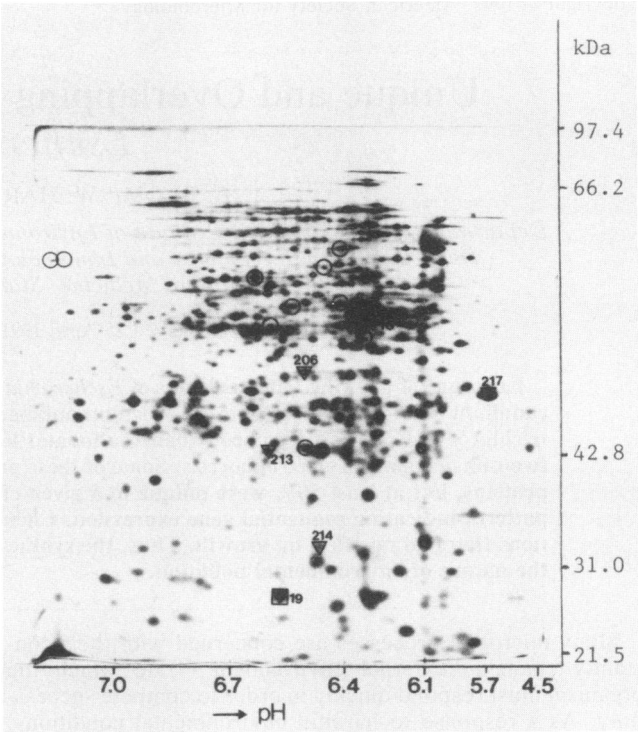

FIG. 1. Two-dimensional autoradiographs of polypeptides synthesized by *E. coli* after 60 min of exposure to PCP. The protein spots unique to this chemical stress (○) are emphasized. Some proteins overlapping with other chemical stresses (▽) and carbon starvation (□) are also indicated. Numbers above 200 indicate previously unreported protein spots. See figures in article by Matin (7) for two-dimensional gel maps of unstressed and starved *E. coli*.

established that all agents altered the protein synthesis pattern (Fig. 1) and that individual stress protein synthesis exhibited a temporal pattern; some were expressed within 15 min and were not synthesized after 60 min of exposure, whereas others appeared later, sometimes as late as 120 or 180 min.

Several of the proteins induced by the chemical treatment have been previously identified in our laboratory as Cst, Pex (i.e., starvation proteins whose induction by starvation is, respectively, cAMP dependent and independent), or heat shock proteins (7); these are indicated by specific numbers (see reference 7 for the numbering scheme of starvation proteins on gel maps) in Tables 2 and 3, which includes all the proteins induced by a given chemical at various times after exposure. Many other proteins were, however, unique to chemical stress. Some of these were commonly induced by more than one chemical (Table 3). For example, protein 217 (Fig. 1) was induced by ether, CPV, HCB-ether, PCP, and TCE. However, 50 to 90% of the proteins induced were unique to a given pollutant (Table 4).

Neither exposure to HCB (0.01 ppm) or benzene (980 ppm) nor culturing in a closed bottle led to any growth inhibition. However, these conditions altered the protein synthesis pattern. Thus, the two-dimensional PAGE protein synthesis profile is a more sensitive indication of stress than growth patterns.

TABLE 2. Overlap of chemical stress proteins with other stress proteins, as judged by comparison of gels with published results (3, 5, 13)

Chemical	Carbon starvation proteins[a]																Heat shock proteins[a]					
	Pex[b]					Cst[b]																
	17	3	38	9	31	35	29	24	27	5	15	25	8	11	72	66	17	38	19	11	49	104
Benzene	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−
$CdCl_2$	+	+	+	+	−	+	+	+	+	−	−	−	−	+	−	−	+	+	+	+	−	−
CPV	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−
DCA	+	+	+	+	−	+	+	+	−	−	−	−	−	−	−	−	+	+	−	−	−	−
DOP	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−
HCB	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	−
PCP	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	+	−
TPBS	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
TCE	+	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	+	−	+	−	+	−
Closed control	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	+

[a] See reference 7 for numbering scheme of starvation proteins on gel maps. Symbols: −, no overlap; +, overlap.
[b] Pex and Cst protein inductions are cAMP independent and dependent, respectively (7).

TABLE 3. Specific and overlapping chemical stress proteins

Protein no.	Overlap with chemical stress protein									Molecular size (kDa)	pH
	Benzene	$CdCl_2$	CPV	DCA	DOP	HCB[a]	PCP	TPBS	TCE		
200	−	+	+	+	−	−	−	−	−	5	7.1
201	+	+	−	+	−	−	−	−	−	59	6.6
202	−	+	−	+	−	−	−	−	−	60	6.6
203	+	+	−	+	−	−	−	−	−	62	6.7
204	−	+	−	+	−	−	−	−	−	64	6.4
205	−	+	−	+	−	−	−	−	−	65	6.5
206	−	+	−	−	−	−	+	−	−	51	6.2
207	−	+	−	−	−	−	−	+	−	58	6.7
27	−	+	−	−	−	+	−	−	−	51	6.4
209	−	−	−	+	−	−	+	−	−	57	7.0
211	−	−	−	+	+	−	−	−	−	42	6.8
212	−	−	+	+	−	−	−	−	−	42	6.3
213	−	−	+	−	+	−	+	−	−	42	6.6
214	−	−	−	−	+	−	+	−	−	32	6.5
216	−	−	−	−	−	+	+	−	−	57	6.5
217	−	−	+	−	−	*	+	−	+	48	5.7
218	−	−	+	−	−	+	−	−	−	57	7.1
219	−	−	+	−	−	+	−	−	+	54	6.4
220	−	−	+	−	−	*	−	−	−	55	6.2
222	−	−	+	−	−	+	−	−	−	38	4.0
223	−	−	+	−	−	+	−	−	−	29	6.1
224	−	−	+	−	−	+	−	−	−	39	6.1

[a] These polypeptides were also induced by ether, which was used as the solvent for HCB. Therefore, it is not known whether HCB induces these polypeptides.

DISCUSSION

We show here that exposure of *E. coli* to several individual pollutants results in the induction of a large number of stress proteins. Some of the proteins were commonly induced by more than one chemical, and others corresponded to heat shock and starvation proteins. However, at least 50% of the induced proteins were unique to a specific chemical. It is noteworthy that about half of the starvation proteins induced by chemical stress belonged to the Pex class (i.e., independent of positive cAMP regulation), which is responsible for general stress resistance in *E. coli* (7, 9). The number of proteins induced by an individual chemical ranged between 13 (for DOP exposure) and 38 (for $CdCl_2$ exposure); a total of 236 different proteins were induced in response to 12 different stress situations tested (including the control experiments). Although 92 of these proteins were induced by more than one stress, no single protein or set of proteins was found as being a universal, nonspecific response to chemical stress. There was a clear temporal pattern to the synthesis of chemical stress proteins, indicating sequential gene expression, as has been observed for starvation (3), heat shock (11, 15), SOS (16), and oxidation stresses (10). No data are available for prolonged chemical stress (i.e., longer than 3 h). During starvation stress, some new proteins are synthesized even after 4 h (5), while a shift in temperature induces heat shock proteins within 1 to 2 min, returning to basal synthesis levels within 15 to 20 min (11). After 120 and 180 min of exposure to CPV, benzene, or TCE, synthesis of new stress proteins was still found in the present study. Thus, it may take from 20 min to 4 h or longer for the cells to adjust their cellular processes to a stressful environment.

It is evident that stress protein synthesis is a more sensitive index of stress than growth rate, since pollutant concentrations at which little or no growth inhibition occurred evoked stress protein synthesis. These results show a promising prospect for stress protein analysis as an alternative and more sensitive method for measuring toxic effects in organisms at sublethal levels (1). Furthermore, the fact that individual chemicals induce unique proteins can conceivably provide a means of identifying pollutants in an environment.

TABLE 4. Number of unique and overlapping proteins induced by chemical pollutants

Chemical	Total no. of proteins induced	No. of proteins common to:			No. of unique proteins
		Carbon starvation proteins	Heat shock proteins	Other chemicals	
Benzene	23	2			21
$CdCl_2$	38	9	4	9	20
CPV	36	3	2	10	23
DCA	34	5	2	9	20
DOP	13	2	1	3	7
HCB	25	4	1	7	11
PCP	30	3	2	6	20
TCE	22	3	3	2	16
TPBS	15	2	1	1	11

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