FEMS Microbiology Letters 111 (1993) 189–196

© 1993 Federation of European Microbiological Societies 0378-1097/93/$06.00
Published by Elsevier

FEMSLE 05537

Transient repression of the synthesis of OmpF and aspartate transcarbamoylase in *Escherichia coli* K12 as a response to pollutant stress

Folkert Faber [a], Thomas Egli [b] and Wim Harder [a]

[a] *Department of Biology, TNO Institute of Environmental Sciences, Delft, the Netherlands, and* [b] *EAWAG, Dübendorf, Switzerland*

(Received 23 March 1993; revision received 3 May 1993; accepted 10 May 1993)

Abstract: The synthesis of total cellular proteins in *Escherichia coli* K12 was studied in batch culture following exposure of cells to low concentrations of monochlorophenol, pentachlorophenol and cadmium chloride. Changes in protein patterns were identified after pulse–chase labelling of proteins with [^{35}S]methionine and subsequent two-dimensional gel electrophoresis (2D-PAGE). We demonstrated that besides the induction of some stress proteins, also a transient decrease in the rate of synthesis of other proteins occurred. Two of these proteins were identified as OmpF and aspartate transcarbamoylase (ATCase). Their transient repression appeared to be a general response to stress elicited by different pollutants and may therefore be used as a general and sensitive early warning system for pollutant stress.

Key words: Escherichia coli K12; Environmental stress; Two-dimensional gel electrophoresis; Stress protein

Introduction

In their natural habitats, microorganisms are frequently exposed to a variety of environmental stresses and these are known to elicit specific responses. Of these, the synthesis of specific stress proteins is most common. The stress proteins induced following exposure to a particular type of environmental stress may belong to either of several stress response systems including the heat shock response [1], the SOS response [2], oxidative stress [3] or starvation response [4]. On the other hand, synthesis of stress proteins may also be specific for one particular stress factor [5]. Induction of stress proteins, general or specific, could be used as a tool in environmental monitoring, because their synthesis may serve as a biological indicator by which the presence of environmental pollutants can be established [5].

So far, research has been concentrating on the induction of stress proteins as an indicator for environmental stress. However, repression of the synthesis of proteins following exposure of cells to stress conditions may also occur [6]. In this paper, we demonstrate that micropollutants can

Correspondence to: W. Harder, TNO Institute of Environmental Sciences, Schoemakerstraat 97, P.O. Box 6011, 2600 JA Delft, the Netherlands.

elicit specific stress protein responses, in which the repression of the synthesis of some proteins seems to be a more general response than induction of stress proteins.

Materials and Methods

Bacterial strains

All bacterial strains used are derivatives of *E. coli* K12. Experimental work was either performed with Stanford *E. coli* K12 [5], strain CE 1241 (F$^-$ *thi ΔphoE proAa / oB pyrF thyA argG ilvA his mal tonA rpsL deoC sup sus uvrB vtr glpR phoR69 phoA8 lambda*$^-$) [7] or strain MA1006 (W. Maas strain; Hfr *pyrB48 thi-1 relA1 lacZ43 spoT1 lambda*$^-$) [8] as indicated in the text.

Media and growth conditions

All strains were grown in batch culture at 37°C in a water bath shaker in 200-ml conical flasks containing 50 ml of medium. For growth and pulse–chase labelling experiments *E. coli* K12 was grown in M9 medium [9] supplemented with 0.4% glucose. At the start of the exponential phase (OD_{660} approx. 0.2), micropollutants were added to final concentrations as indicated in the text. The chemicals used were monochlorophenol (MCP), pentachlorophenol (PCP) and cadmium chloride (stock solutions concentrated 1000×, MCP and PCP dissolved in ethanol, final ethanol concentration in the cultures was 0.1% v/v). Cultures without micropollutants or with 0.1% (v/v) ethanol were used as controls. Growth was monitored by measuring OD at 660 nm. Samples were also taken for measuring the rate of oxygen uptake of cell suspensions using a Biological Oxygen Monitor (Yellow Springs Instruments, Model 5300).

Strain CE1241 was grown in YEPD medium (1% yeast extract, 1% bacto-peptone, 2% glucose). Strain MA1006 was grown in M9 medium supplemented with 0.4% glucose, 20 mg l^{-1} uracil and 15 mg l^{-1} thiamine.

Analysis of cellular proteins

Pulse–chase labelling experiments were performed to investigate the rate of synthesis of individual cellular proteins. Proteins were labelled and separated by 2D-PAGE as described by Groat et al. [4]. Labelled proteins were visualized by autoradiography (XAR-5 film; Eastman Kodak). Comparison of the autoradiograms was carried out both visually and with the image analysis system of the Millipore Corporation (Bio Image Electrophoresis Analyzer, Model 60S 2-D System). This system was also used to quantify individual protein spots on autoradiograms. The numbers given to the proteins are arbitrary. Total protein patterns of samples were identified by 2D-PAGE [4] and subsequent silver staining of gels according to Morrisey [10].

Table 1

Effect of micropollutants on the growth rate of *E. coli* K12, expressed as a percentage of the doubling time (DT) of the control cultures

Monochlorophenol		Pentachlorophenol		Cadmium chloride	
Conc. [a] (mg l^{-1})	Doubling time (%)	Conc. [a] (mg l^{-1})	Doubling time (%)	Conc. [a] (mg l^{-1})	Doubling time (%)
0.0	100	0.0	100	0.0	100
2.0	99	0.01	99	0.01	99
20.0	99	0.1	99	0.1	98
100.0	89	1.0	97	1.0	88
200.0	81	10.0	91	10.0	68
500.0	– [b]	50.0 [c]	61	50.0	44

[a] Actual concentrations of the chemicals in the cultures were not measured. The figures given are calculated concentrations.
[b] Culture stopped growing after addition of 500 mg l^{-1} MCP.
[c] Solubility of PCP in water at 37°C is about 23 mg l^{-1} [12].

Results

Growth experiments

E. coli K12 cultures were exposed to various concentrations of the three chemicals MCP, PCP and $CdCl_2$ and the growth rate was determined. The doubling times (DT) of these exposed cultures are presented in Table 1 as a percentage of the DT measured in control cultures (without pollutants or in the presence of 0.1% (v/v) ethanol) which was 113 ± 3 min. Addition of 100 mg l^{-1} MCP, 10 mg l^{-1} PCP and 1 mg l^{-1} $CdCl_2$ to cultures resulted in an inhibition of growth, which was to the same extent as that published in the literature [11,12].

Cultures exposed to 200 mg l^{-1} MCP or 0.1 mg l^{-1} $CdCl_2$ showed no change in the rate of oxygen uptake compared to the controls. Addition of PCP to cultures resulted in an increase in the rate of oxygen uptake, which was higher at 10 mg l^{-1} PCP (66 μmol l^{-1} min^{-1} OD_{660}^{-1}) than at 1 mg l^{-1} PCP (33 μmol l^{-1} min^{-1} OD_{660}^{-1}). This

Table 2

Changes in protein patterns of *E. coli* K12 after exposure to micropollutants

Micropollutant	Number of induced proteins at sampling time (min)				Number of repressed proteins at sampling time (min)			
	3	30	60	120	3	30	60	120
$CdCl_2$ (0.1 mg l^{-1})	29	32	18	9	19	31	16	5
PCP (10 mg l^{-1})	– [a]	9	15	3	[a]	19	12	9
MCP (200 mg l^{-1})	– [a]	28	27	23	[a]	41	20	27

[a] No samples taken for pulse–chase labelling.

Table 3

Selected proteins induced or repressed by 200 mg l^{-1} MCP, 10 mg l^{-1} PCP or 0.1 mg l^{-1} $CdCl_2$ in cultures of *E. coli* K12

Protein no.	Location [a]	MCP			PCP			$CdCl_2$			
		30 min	60 min	120 min	30 min	60 min	120 min	3 min	30 min	60 min	120 min
1	24× 24	++	++	++	+	+		+	+	+	
3	68×116	+	+	+					+		
4	53×113	−−	−−	−							
7	115× 80	−−	−−	−−	−−	−	−		−	−	−
8	118× 93	−	−	−							
9	26× 77	−−	−−	−−	−	−			−		
15	36× 71	+	+	+	−	−	−				
34	48× 39							+	++		
37	39× 99			−			−		++		
39	59× 99							+	++	+	
40	61×114	−−	−−	−		−	−		−−		
42	78×111						−	+	++		
44	75×120	−−	−−	−−					−		
46	64×100	−	−−	−−	−			+	++		
51	80× 54	−	−	−					+		
54	64× 71										++

++, strongly induced; +, induced; −, repressed; −−, strongly repressed.

[a] Each protein spot is located on the reference autoradiogram in Fig. 1A by its coordinates (abscissa×ordinate), according to Phillips et al. [13].

increase in oxygen uptake is probably caused by uncoupling.

Protein patterns in E. coli after exposure to micropollutants

The concentrations of micropollutants used in pulse–chase labelling experiments were 200 mg l^{-1} MCP, 10 mg l^{-1} PCP and 0.1 mg l^{-1} $CdCl_2$. At these concentrations the cultures exhibited slight growth inhibition and therefore some effect on the pattern of protein synthesis was expected. The total rate of protein synthesis was hardly affected by the conditions used, since total incorporation of [^{35}S]methionine was approximately the same for control and exposed cultures (results not shown). The temporal changes in the rate of synthesis of individual cellular proteins were identified by comparing the 2D-PAGE patterns of exposed cultures to their controls. The total protein patterns did not change as significantly as has been reported for starvation experiments [4], but some distinct differences were observed. Only a few proteins were newly induced or completely repressed. For many proteins the rate of synthesis was only partly affected. Table 2 lists the number of proteins for which major changes were observed in their rate of synthesis (at least two-fold increase or decrease).

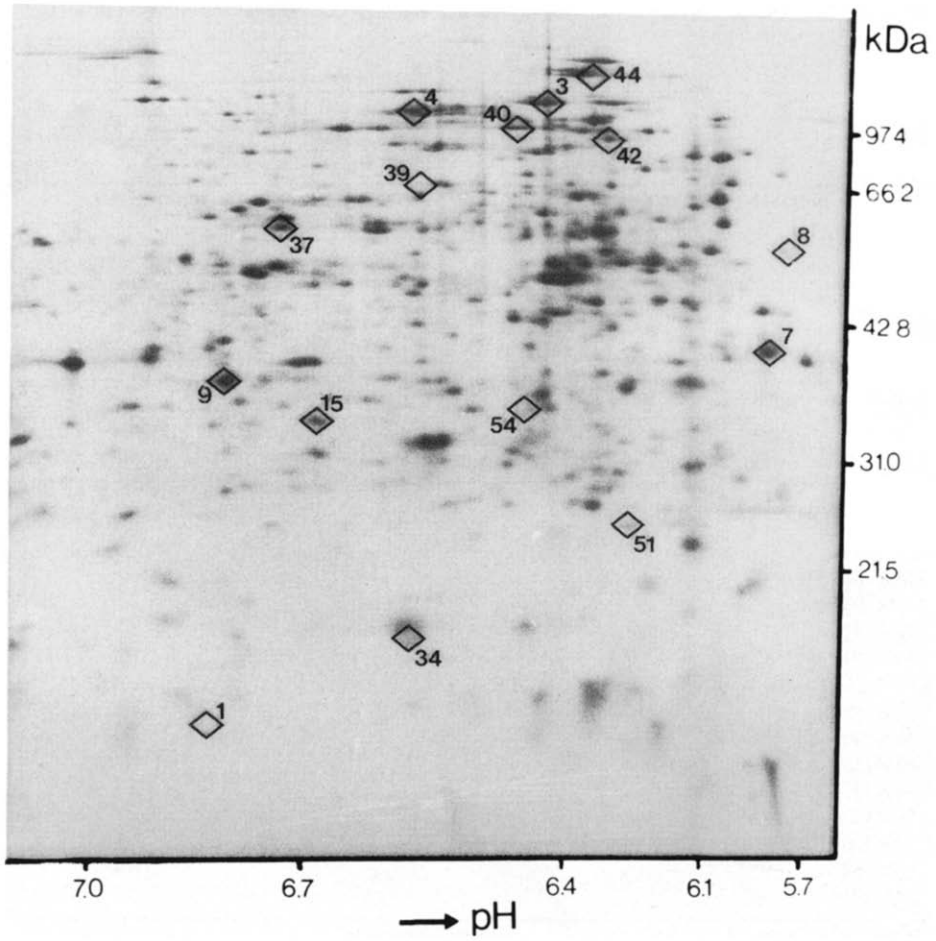

Fig. 1. Two-dimensional autoradiogram of exponentially growing *E. coli* K12 showing proteins synthesized in the absence of any stress factor. Proteins selected for detailed analysis are marked and listed in Table 3.

Most of the proteins listed in Table 2 were induced or repressed only at one particular time, indicating that it was a transient response. Most changes occurred within the first 30 min. The proteins listed in Table 3 were considered to be of sufficient interest to warrant a more detailed investigation. These proteins showed a clear and prolonged change in the rate of synthesis (factor 3–35) compared to the controls. Figure 1 shows the location of these proteins on a reference autoradiogram. Except for protein no. 1, no other generally induced protein was detected. The synthesis of proteins nos. 7, 9 and 40 was repressed by all micropollutants, although there were considerable differences in the degree of repression. The synthesis of only a few proteins was specifically changed by the micropollutants tested. Proteins nos. 4 and 8 were repressed by MCP and proteins nos. 34, 39 and 54 were induced by $CdCl_2$, exclusively. In some cases the effect on the synthesis of a protein differed with respect to the micropollutant used. Protein 15 was induced by MCP, but slightly repressed by PCP. Proteins 37, 42, 46 and 51 were repressed by MCP and/or PCP, but induced by $CdCl_2$.

The data suggest that repression of the synthesis of certain proteins is at least as frequent or even more general a response to low concentrations of pollutants than induction (Table 3). These results confirm and extend the work reported by Blom et al. [5], where nine micropollutants were screened for the induction of stress proteins in *E. coli* K12. They detected no generally induced stress proteins. However, of the nine micropollutants tested, six also repressed the synthesis of protein no. 9, while four of the micropollutants used also repressed the synthesis of protein no. 7 (A.J.M. Blom, unpublished results). Because previous investigations concentrated on induced (stress) proteins, we selected repressed proteins nos. 7 and 9 for a more detailed study.

Identification of selected proteins

On the basis of the location in a reference autoradiogram ([13]; p. 920, Fig. 1A), we tentatively identified protein no. 7 as OmpF (115 × 80). This was confirmed by an *E. coli* K12 mutant lacking OmpF (CE1241) [7], which lacked the protein spot at this location in a 2D-gel (Fig. 2B). Positive identification of protein no. 7 was obtained immunologically by using monoclonal antibodies against OmpF (results not shown).

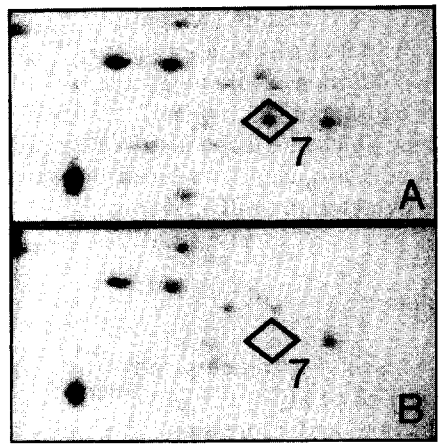

Fig. 2. Two-dimensional gels of exponentially growing *E. coli* K12 Stanford strain (**A**) and CE 1241 (an *E. coli* K12-mutant, lacking OmpF) (**B**). Only part of the silver-stained gels are shown, focused at the location of OmpF.

Protein no. 9 was tentatively identified as ATCase (26 × 77) on the basis of its location in the reference autoradiogram [13]. Strain MA1006, an

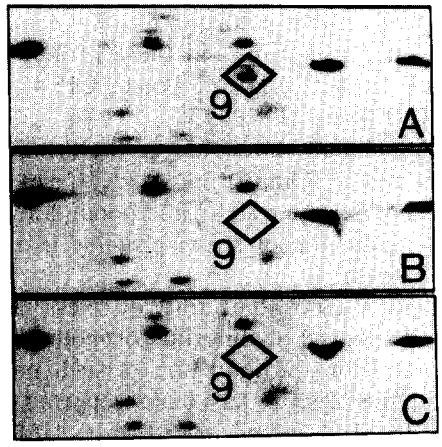

Fig. 3. Two-dimensional gels of exponentially growing *E. coli* K12 Stanford strain (**A**), MA1006 (an *E. coli* K12-mutant lacking aspartate transcarbamoylase (ATCase)) and *E. coli* K12 Stanford strain, cultured with 20 mg l^{-1} uracil (**C**). Only part of the silver-stained gels are shown, focused at the location of ATCase.

E. coli K12 *pyrB* mutant lacking ATCase, showed no protein spot at this location in the 2D-gel (Fig. 3B). Also, addition of uracil (20 mg l^{-1}) to a M9 medium culture of *E. coli* K12 Stanford strain, conditions which are known to repress the synthesis of ATCase, clearly reduced the amount of ATCase detected on a 2D-gel (Fig. 3C).

Discussion

Our present experiments show that exposure of *E. coli* K12 to MCP, PCP and $CdCl_2$ results in the induction and repression of a considerable number of proteins. The observed changes in protein patterns were clearly time-dependent and most of the proteins were induced or repressed only transiently. This temporal effect has also been observed for heat shock [1].

Although cadmium is known to induce some of the heat shock proteins, the extent of induction in our experiments was not as strong as has been reported in the literature [14]. This is probably due to the low concentration (0.1 mg l^{-1}) of $CdCl_2$ used in the present work. From their location on a 2D-gel, the induced heat shock proteins we identified were C15.4, C62.5, D33.4 and H94.0 [13]. The observed induction was approximately two-fold 30 min after addition of $CdCl_2$. MCP also showed a slight induction of some heat shock proteins at $t = 30$ min, and these proteins were identified as B25.3, B56.5, B66.0, C15.4, C62.5, D33.4, G21.0 and H94.0 [13].

We found that repression of the synthesis of proteins nos. 7 (OmpF) and 9 (ATCase) was a more general response to micropollutant stress than the induction of stress proteins in this *E. coli* K12 strain.

To date, the possible utility of stress proteins for the biological monitoring of pollution has concentrated on the induction of such proteins. For example, Hsp70 has been reported to be induced by Pb^{2+} in soil invertebrates [15], while Hsp60 is induced by Cu^{2+} in blue mussels [16]. We have demonstrated in this study that the transient repression of the synthesis of proteins might also serve as a tool for assessing stress conditions in the environment.

Acknowledgements

This work was supported by the Netherlands Organization for Applied Scientific Research. We are indebted to A.J.M. Blom, P.M. Houpt, J.W. Vonk, R.F.M. van Gorcom and Millipore Corporation for their helpful assistance and discussions. We also thank J. Tommassen for the CE 1241-strain and the monoclonal antibodies against OmpF. T. Egli thanks EERO for a short-term fellowship.

References

1 Lindquist, S. (1988) The heat-shock proteins. Annu. Rev. Genet. 22, 631–677.
2 Walker, G.C. (1984) Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. 48, 60–93.
3 Morgan, B.W., Christman, M.F., Jacobson, F.S., Storz, G. and Ames, B.N. (1986) Hydrogen peroxide-inducible proteins in *Salmonella typhimurium* overlap with heat shock and other stress proteins. Proc. Natl. Acad. Sci. USA 83, 8059–8063.
4 Groat, R.G., Schultz, J.E., Zychlinsky, E., Bockman, A. and Matin, A. (1986) Starvation proteins in *Escherichia coli*: kinetics of synthesis and role in starvation survival. J. Bacteriol. 168, 486–493.
5 Blom, A., Harder, W. and Matin, A. (1992) Unique and overlapping pollutant stress proteins of *Escherichia coli*. Appl. Environ. Microbiol. 58, 331–334.
6 Neidhardt, F.C. and VanBogelen, R.A. (1987) Heat shock response. In: *Escherichia coli* and *Salmonella typhimurium* Cellular and Molecular Biology (F.C. Neidhardt, Ed.), pp. 1335–1345. American Society for Microbiology, Washington, DC.
7 Korteland, J., Tommassen, J. and Lugtenberg, B. (1982) PhoE protein pore of the outer membrane of *Escherichia coli* K12 is a particularly efficient channel for organic and inorganic phosphate. Biochim. Biophys. Acta 690, 282–289.
8 Beckwith, J.R., Pardee, A.B., Austrian, R. and Jacob, F. (1962) Coordination of the synthesis of the enzymes in the pyrimidine pathway of *E. coli*. J. Mol. Biol. 5, 618–634.
9 Reeve, C.A., Bockman, A.T. and Matin, A. (1984) Role of protein degradation in the survival of carbon-starved *Escherichia coli* and *Salmonella typhimurium*. J. Bacteriol. 157, 758–763.
10 Morrisey, J.H. (1981) Silver stain for proteins in polyacrylamide gels: A modified procedure with enhanced uniform sensitivity. Anal. Biochem. 117, 307–310.
11 Hommel, D.G. (1987) Handbuch der gefährlichen Güter (4th edn.), Springer-Verlag, Berlin, Heidelberg.
12 Verschueren, K. (1983) Handbook of Environmental Data on Organic Chemicals. Van Nostrand Reinholt Company.

13 Phillips, T.A., Vaughn, V., Bloch, P.L. and Neidhardt, F.C. (1987) Gene-protein index of *Escherichia coli* K-12. In: *Escherichia coli* and *Salmonella typhimurium* Cellular and Molecular Biology (F.C. Neidhardt, Ed. in chief), 2nd edn., pp. 919–966. American Society for Microbiology, Washington, DC.

14 VanBogelen, R.A., Kelley, P.M. and Neidhardt, F.C. (1987) Differential induction of heat shock, SOS, and oxidation stress regulons and accumulation of nucleotides in *Escherichia coli*. J. Bacteriol. 169, 26–32.

15 Köhler, H.-R., Triebskorn, R., Stöcker, W., Kloetzel, P.-M. and Alberti, G. (1992) The 70 kD heat shock protein (hsp70) in soil invertebrates: A possible tool for monitoring environmental toxicants. Arch. Environ. Contam. Toxicol. 22, 334–338.

16 Sanders, B.M. and Martin, L.S. (1991) Relationships between accumulation of a 60 kDa stress protein and scope-for-growth in *Mytilus edulis* exposed to a range of copper concentrations. Marine Environ. Res. 31, 81–97.