JOURNAL OF BACTERIOLOGY, Nov. 1993, p. 7105–7108
0021-9193/93/217105-04$02.00/0

Vol. 175, No. 21

Adaptation of *Escherichia coli* to the Uncoupler of Oxidative Phosphorylation 2,4-Dinitrophenol

DANIEL J. GAGE† AND FREDERICK C. NEIDHARDT*

Department of Microbiology and Immunology and Program in Cellular and Molecular Biology, University of Michigan Medical School, 6643 Medical Science Building II, Ann Arbor, Michigan 48109-0620

Received 3 June 1993/Accepted 1 September 1993

***Escherichia coli* was found to adapt to the uncoupler of oxidative phosphorylation 2,4-dinitrophenol. The rates of synthesis of 53 proteins were increased following exposure to 2,4-dinitrophenol. Adaptation was accelerated when the cofactor pyrroloquinoline quinone was provided in the growth medium.**

In *Escherichia coli*, energy associated with the oxidation of an energy source is conserved as an electrochemical potential difference of hydrogen ions across the cytoplasmic membrane and is termed the proton motive force (Δp). The electrical potential ($\Delta\Psi$) together with the difference in proton concentration between the periplasm and cytoplasm (ΔpH) make up the proton motive force, which is typically maintained at a steady-state value of -150 to -200 mV (14). Δp is harnessed to drive nutrient transport, ATP synthesis, and other endergonic reactions at the cell membrane.

Uncouplers of oxidative phosphorylation, such as 2,4-dinitrophenol (DNP) and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), are able to transport protons across biological membranes, which usually have extremely low proton conductance (16, 17). By transporting protons down the electrochemical potential gradient, these agents collapse Δp. The collapse of Δp has detrimental effects on the bacterial cell: (i) the rate of proton-coupled ATP synthesis declines; (ii) the pH of the cytoplasm, typically maintained near 7.8 (3), approaches that of the external medium (8); and (iii) nutrient transport is inhibited, as is the translocation of proteins destined for insertion into the cytoplasmic membrane (1, 7, 10).

Here we show that wild-type *E. coli* is able to adapt to the uncoupler of oxidative phosphorylation DNP and that the rate of adaptation is accelerated by the cofactor pyrroloquinoline quinone (PQQ). In addition, we show that *E. coli* induces approximately 50 proteins, including the heat shock proteins, upon exposure to DNP.

***E. coli* adapts to DNP.** *E. coli* W3110 growing in glucose-rich morpholinepropanesulfonic acid (MOPS) medium (GRM) without methionine (9) at 28°C was exposed to 0.5, 0.75, and 1.0 mM DNP. Following challenge with these concentrations of DNP, there were immediate cutbacks in the growth rate of 45, 75, and 90%, respectively (Fig. 1). Following the cutback in growth rate, cultures exposed to 0.5 and 0.75 mM DNP increased their growth rates and nearly reached the rate at which they grew before exposure to DNP. The growth rate of the culture exposed to 1.0 mM DNP increased somewhat but never reached the pre-DNP growth rate. A similar growth response has been noted when *E. coli* is exposed to inhibitory concentrations of CCCP (18).

PQQ accelerates the adaptation of *E. coli* to DNP. Wild-type *E. coli* cells are able to synthesize the apoenzyme form of glucose dehydrogenase (12). They are unable to synthesize PQQ, the cofactor for this enzyme, under any known condition (2, 12). Under aerobic conditions in the presence of added PQQ, glucose dehydrogenase catalyzes the oxidation of glucose to gluconate, with the resulting electrons entering the electron transport chain (15). Thus, glucose dehydrogenase can contribute to energy production in *E. coli* by generating Δp (15, 22). The apoenzyme form of glucose dehydrogenase is induced 3- to 15-fold following exposure to 1.0 mM DNP (12). In light of these results, strain W3110 grown in GRM without methionine at 28°C was challenged with 1.0 mM DNP in the presence or absence of 20 μM PQQ. The culture which received PQQ recovered quickly from the DNP-induced growth lag (Fig. 2). PQQ did not increase the growth rate of a culture growing in medium without DNP, indicating that PQQ did not act as a general growth enhancer. It was shown that glucose dehydrogenase, and not some other enzyme which utilizes PQQ as a cofactor, was responsible for the accelerated adaptation to DNP by conducting the same experiment using isogenic strain DG300 (*gcd::cat*), which contains a chloramphenicol resistance marker inserted into the gene for glucose dehydrogenase (4). This mutant failed to recover quickly from DNP challenge, even in the presence of exogenous PQQ. It was possible that it was not the energy derived from the oxidation of glucose which increased the growth rate of cells exposed to DNP, but rather the generation and utilization of gluconate (0.4% [wt/vol]). This possibility was ruled out by the observation that cells grown with glucose and gluconate together had no growth advantage over cells growing in glucose alone when they were exposed to DNP (data not shown).

It is likely that the energy generated by glucose dehydrogenase is beneficial when *E. coli* is exposed to DNP. This result indicates that energy production may be the rate-limiting process for growth when cells are challenged with this agent. This possibility supports the suggestion that one function of *E. coli* glucose dehydrogenase is to supply energy under conditions where it is limiting, provided PQQ is present (13).

***E. coli* induces proteins following exposure to DNP.** Strain W3110 grown in GRM without methionine at 28°C was pulse-labeled for 5 min before and for 15 to 20 min after exposure to 0.5 mM DNP. Analysis by two-dimensional gel electrophoresis revealed that the rates of synthesis of 53 proteins were induced more than approximately twofold by DNP (Fig. 3 and Table 1) (9). These proteins included all of the heat shock proteins except LysU, HtpE, and HtpN. Among the non-heat shock proteins induced by DNP, 6 were also induced by carbon starvation, 9 were also induced by nitrogen starvation, and 11 were also induced by phosphate starvation

* Corresponding author.
† Present address: Department of Biological Sciences, Stanford University, Stanford, CA 94305-5020.

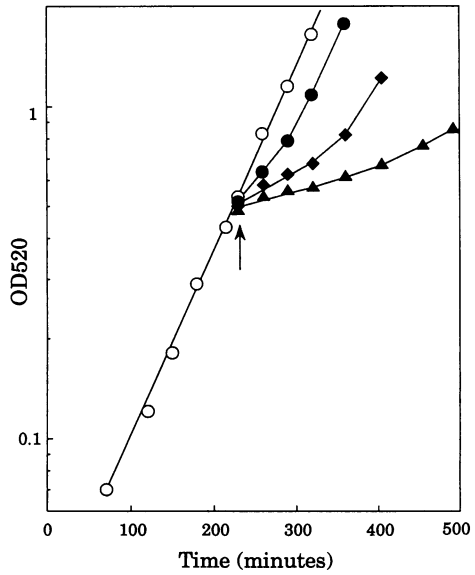

FIG. 1. Growth of strain W3110 after exposure to DNP. Growth of strain W3110 was monitored in GRM without methionine at 28°C (○) and in the same medium containing 0.5 (●), 0.75 (◆), or 1.0 (▲) mM DNP. OD520, optical density at 520 nm.

(Table 1). Of the 53 proteins, 24 have not been found to be induced by any other stress and thus are, so far, unique to the DNP stimulon. When pulse-labeling was done after cells had adapted to DNP, the analysis revealed that the spectrum of proteins being synthesized was very similar to that seen during steady-state growth prior to DNP addition (data not shown).

While it is not known how DNP induces the heat shock proteins, it is tempting to speculate that it does so by inhibiting the maturation and translocation of proteins normally destined to be inserted into or transported across the cytoplasmic membrane. When translocation is inhibited, the cellular systems for chaperoning these proteins to the cell surface may be

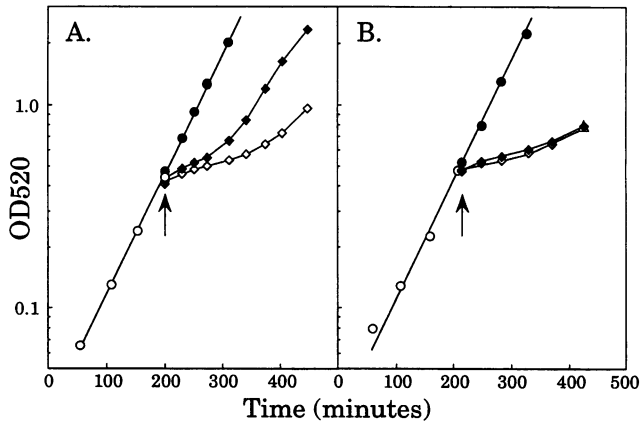

FIG. 2. PQQ accelerates recovery from a DNP-induced growth lag. Cells growing in GRM without methionine (○) at 28°C were transferred to a flask containing either PQQ (●), DNP (◇), or PQQ and DNP (◆). PQQ was present at a final concentration of 20 μM, and DNP was present at a final concentration of 1.0 mM. Results with wild-type strain W3110 (A) and isogenic strain DG300 (*gcd*::*cat*) (B) are shown. OD520, optical density at 520 nm.

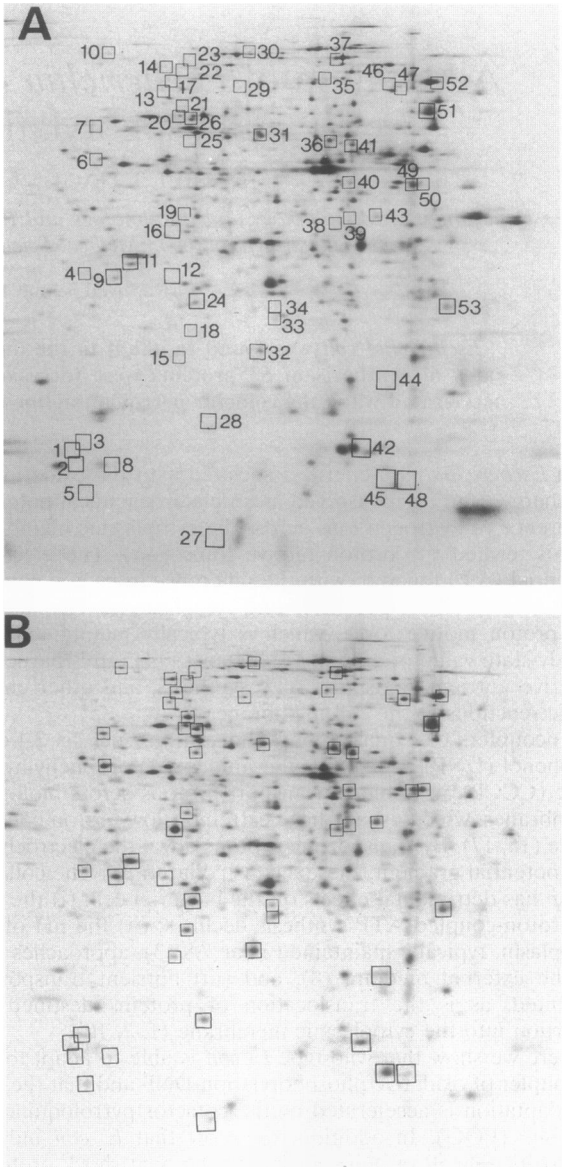

FIG. 3. Induction of proteins following exposure to DNP. Strain W3110 growing in GRM without methionine was labeled with [^{35}S]methionine before (A) and 15 to 20 min after the addition of DNP (B). Proteins whose rates of synthesis increased more than twofold are boxed. The numbers correspond to the protein numbers enumerated in Table 1.

overwhelmed by the increase in the intercellular concentration of precursor proteins. This situation may result in binding of these proteins by the heat shock chaperones. Once the chaperones are occupied, there would be an increase in the half-life and hence the level of σ^{32}, the heat shock sigma factor (20). This model is similar to models proposed to explain induction of the heat shock regulon following an increase in temperature (5). In support of this type of model, it has been shown that uncouplers can inhibit the translocation of precursor proteins (7) and that Δp is required for protein translocation (6, 10, 19). In addition, it has recently been shown that the accumulation of precursor proteins can induce a heat shock response and that this induction is probably the result of an increase in the

TABLE 1. Proteins induced in *E. coli* by 0.5 mM DNP

Protein no.	Coordinates[a]	Alphanumeric designation[b]	Gene	Protein	Inducing conditions[c]
1	23 × 32	H15.9			
2	24 × 29	H15.6			
3	24 × 33	H16.0			
4	25 × 70	H30.8			
5	26 × 25	H15.0			
6	28 × 94	H50.4	*fum*	Fumarase	C
7	28 × 100	H54.7			
8	30 × 30	H15.65			
9	31 × 68	H29.5			
10	31 × 115	H94.0	*lon*	Lon protease	Hsp
11	34 × 71	H31.5			
12	40 × 68	G29.4			N, P
13	41 × 107	G67.0			
14	41 × 112	G80.1			Heat, Cd, HP, Ile, QN
15	42 × 50	G21.0	*htpO*	HtpO	Hsp
16	42 × 79	G36.7			
17	43 × 109	G72.1			Lrp, C, P
18	45 × 56	G23.4			P
19	45 × 83	G38.2			N, P
20	45 × 102	G57.6			
21	45 × 104	G60.1			N, P, C
22	45 × 112	G80.5			
23	45 × 114	G88.0			C
24	46 × 63	G26.3			
25	46 × 96	G48.0			N, P
26	47 × 101	G58.5			
27	49 × 14	G10.7			P
28	49 × 37	G16.2			
29	54 × 108	G42.1			N, P
30	55 × 115	G95.0	*ptr*	Protease III	
31	57 × 98	F48.8	*zwf*	Glucose-6-phosphate dehydrogenase	
32	58 × 51	F21.5	*clpP*	Proteolytic subunit of ATP-dependent protease	Hsp
33	59 × 59	F22.5	*eda*	Gluconate aldolase	N, P
34	59 × 61	F25.1			
35	70 × 109	E72.0	*clpB**	Regulatory subunit of ATP-dependent protease	Hsp
36	71 × 97	E48.7	*glpK*	Glycerol kinase	
37	72 × 114	F84.0	*clpB*	Regulatory subunit of ATP-dependent protease	Hsp
38	73 × 79	D33.4	*htpH*	HtpH	Hsp
39	75 × 79	D35.7			N, Cd, QN
40	76 × 88	D40.7	*livJ*	LIV-binding protein	Lrp
41	77 × 95	D48.5	*htpI*	HtpI	Hsp
42	79 × 30	C15.4	*mopB*	GroES	Hsp
43	81 × 80	C36.3			P
44	84 × 46	C18.1			
45	85 × 24	C13.5	*uspA*	Universal stress protein	Heat, C, N, P, HP, Ile
46	85 × 107	C67.0			
47	87 × 107	C62.5	*htpG*	HptG	Hsp
48	89 × 24	C13.4	*uspA*	Universal stress protein	Heat, C, N, P, HP, Ile
49	93 × 88	B40.8	*livK*	Leucine-binding protein	
50	95 × 88	C42.9			
51	102 × 102	B56.5	*mopA*	GroEL	Hsp
52	104 × 108	B66.0	*dnaK*	DnaK	Hsp
53	107 × 62	B25.3	*grpE*	GrpE	Hsp

[a] Coordinates indicate the location of the protein on a W3110 reference gel (21).

[b] The assignment of the alphanumeric designation is described elsewhere (21). The letters reflect the pI range of the protein, while the numbers reflect the molecular mass of the protein in kilodaltons.

[c] Abbreviations indicate regulons to which the protein belongs or conditions known to induce the protein (21). Abbreviations are as follows: C, N, and P, proteins induced by carbon, nitrogen, and phosphate starvation, respectively; Hsp, proteins belonging to the heat shock regulon; LIV-binding protein, leucine-isoleucine valine-binding protein; Lrp, proteins under the control of the leucine response regulatory protein (*lrp*); Heat, proteins not in the heat shock regulon which are induced by a temperature shift; Cd, HP, and QN, proteins induced by cadmium chloride, hydrogen peroxide, and the quinone ACDQ (6-amino-7-chloro-5,8-dioxoquinoline), respectively; Ile, proteins induced by isoleucine starvation.

half-life of σ^{32} (23). Under special conditions, bacteria can grow with a Δp which approaches zero (11, 18). The heat shock proteins and translocation system may play an important role in growth under such conditions.

It is easy to imagine that *E. coli* might be exposed to conditions which are mimicked, at least in part, by exposure to DNP. These conditions include the following: (i) a low external pH which would acidify the cytoplasm; (ii) a high concentration of metabolic end products, such as acetate, which are capable of acting as uncouplers; and (iii) exposure to polymixins, colicins, defensins, and other molecules produced by competing organisms, which can compromise the integrity of bacterial membranes. Thus, the study of adaptation to DNP may shed some light on how *E. coli* modifies cellular processes in order to survive and grow when faced with these common types of environmental challenges.

We thank Anne-Marie Cleton-Jansen for the gift of strain PP241 (*gcd*::*cat*).

This work was supported by research grant GM17892 from the National Institute of General Medical Sciences (F.C.N.).

REFERENCES

1. **Bakker, E. P., and L. L. Randall.** 1984. The requirement for energy during export of β-lactamase in Escherichia coli is fulfilled by the total protonmotive force. EMBO J. **3:**895–900.
2. **Biville, F., E. Turlin, and F. Gasser.** 1991. Mutants of *Escherichia coli* producing pyrroloquinoline quinone. J. Gen. Microbiol. **137:** 1775–1782.
3. **Booth, I. R.** 1985. Regulation of cytoplasmic pH in bacteria. Microbiol. Rev. **49:**359–378.
4. **Cleton-Jansen, A., N. Goosen, O. Fayat, and P. Van de Putte.** 1990. Cloning, mapping, and sequencing of the gene encoding *Escherichia coli* glucose dehydrogenase. J. Bacteriol. **172:**6308–6315.
5. **Craig, E. A., and C. A. Gross.** 1991. Is hsp70 the cellular thermometer? Trends. Biochem. Sci. **16:**135–140.
6. **Driessen, A. J., and W. Wickner.** 1991. Proton transfer is rate-limiting for translocation of precursor proteins by the *Escherichia coli* translocase. Proc. Natl. Acad. Sci. USA **88:**1–5.
7. **Enequist, H. G., T. R. Hirst, S. Harayama, S. J. Hardy, and L. L. Randall.** 1981. Energy is required for maturation of exported proteins in *Escherichia coli*. Eur. J. Biochem. **116:**227–233.
8. **Foster, J. W., and H. K. Hall.** 1991. Inducible pH homeostasis and the acid tolerance response of *Salmonella typhimurium*. J. Bacteriol. **173:**5129–5135.
9. **Gage, D. J., and F. C. Neidhardt.** 1993. Modulation of the heat shock response by one-carbon metabolism in *Escherichia coli*. J. Bacteriol. **175:**1961–1970.
10. **Geller, B. L.** 1991. Energy requirements for protein translocation across the *Escherichia coli* inner membrane. Mol. Microbiol. **5:**2093–2098.
11. **Harold, F. M., and J. Van Brunt.** 1977. Circulation of H^+ and K^+ is not obligatory for bacterial growth. Science **197:**372–373.
12. **Hommes, R. W. J., P. W. Postma, O. M. Neijssel, D. W. Tempest, P. Dokter, and J. A. Duine.** 1984. Evidence of a quinoprotein glucose dehydrogenase apoenzyme in several strains of *Escherichia coli*. FEMS Microbiol. Lett. **24:**329–333.
13. **Hommes, R. W. J., B. van Hell, P. W. Postma, O. M. Neijssel, and D. W. Tempest.** 1985. The functional significance of glucose dehydrogenase in Klebsiella aerogenes. Arch. Microbiol. **143:**163–168.
14. **Kashket, E. R.** 1985. The proton motive force in bacteria: a critical assessment of methods. Annu. Rev. Microbiol. **39:**219–242.
15. **Matsushita, K., M. Nonobe, E. Shinagawa, O. Adachi, and M. Ameyama.** 1987. Reconstitution of pyrroloquinoline quinone-dependent D-glucose oxidase respiratory chain of *Escherichia coli* with cytochrome *o* oxidase. J. Bacteriol. **169:**205–209.
16. **McLaughlin, S.** 1972. The mechanism of action of DNP on phospholipid bilayer membranes. J. Membr. Biol. **9:**361–372.
17. **Mitchell, P.** 1966. Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. Biol. Rev. **41:**445–502.
18. **Ohyama, T., S. Mugikura, M. Nishikawa, K. Igarashi, and H. Kobayashi.** 1992. Osmotic adaptation of *Escherichia coli* with a negligible proton motive force in the presence of carbonyl cyanide *m*-chlorophenylhydrazone. J. Bacteriol. **174:**2922–2928.
19. **Schiebel, E., A. J. M. Driessen, F. Hartl, and W. Wickner.** 1991. $\Delta\mu_{H^+}$ and ATP function at different steps of the catalytic cycle of preprotein translocase. Cell **64:**927–939.
20. **Straus, D., W. Walter, and C. A. Gross.** 1990. DnaK, DnaJ, and GrpE heat shock proteins negatively regulate heat shock gene expression by controlling the synthesis and stability of σ^{32}. Genes Dev. **4:**2202–2209.
21. **VanBogelen, R. A., P. Sankar, R. L. Clark, J. A. Bogan, and F. C. Neidhardt.** 1992. The gene-protein database of Escherichia coli: edition 5. Electrophoresis **13:**1014–1054.
22. **Van Schie, B. J., K. J. Hellingwerf, J. P. Van Dijken, M. G. L. Elferink, J. M. Van Dijl, J. G. Kuenen, and W. N. Konings.** 1985. Energy transduction by electron transfer via a pyrrolo-quinoline quinone-dependent glucose dehydrogenase in *Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinetobacter calcoaceticus* (var. *lwoffi*). J. Bacteriol. **163:**493–499.
23. **Wild, J., W. A. Walter, C. A. Gross, and E. Altman.** 1993. Accumulation of secretory protein precursors in *Escherichia coli* induces the heat shock response. J. Bacteriol. **175:**3992–3997.