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EXPERIMENTAL ARTICLES

Changes in the Topological State of DNA during *Escherichia coli* Adaptation to Oxidative Stress under Glucose Starvation and after the Transition to Growth

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Abstract—Changes in the topological state of DNA occur in a starving *Escherichia coli* culture under oxidative stress caused by the addition of hydrogen peroxide. The addition of a carbon and energy source to this culture results in a second stress reaction. This supports previous data indicating that different mechanisms are responsible for the cell defense against oxidative stress in exponential and starving *E. coli* cultures. Polyamine synthesis is involved in the cell adaptation to the stress. Putrescine binding to DNA and its dissociation seem to modulate the DNA topological state, which regulates the expression of the adaptive genes. An increase in the activity of the polyamine-synthesizing system in response to oxidative stress leads to a putrescine flux across the cytoplasmic membrane, due to which the antioxidant activity of putrescine protects the membrane phospholipids and contributes to the restoration of the cell energy-generating function.

Key words: changes in the topological state of DNA, oxidative stress, putrescine, *Escherichia coli*.

Transitional metabolic states are characteristic of natural microbial populations affected by changes in environmental conditions and stress factors. Bacteria display complex adaptive reactions in response to adverse environmental conditions in order to survive various combinations of stress factors. The most frequent combination of adverse factors, especially for aerobic or facultatively anaerobic microorganisms such as *Escherichia coli*, is the combination of starvation and oxidative stress.

Superoxide radical and hydrogen peroxide, the products of one-electron transport in the respiratory chain, are the most frequently occurring causative agents of oxidative stress; during evolution, appropriate defense mechanisms developed in *E. coli* against these agents. The SoxRS regulon is responsible for the defense reaction to the stress caused by superoxide radical [1], whereas the defense against H_2O_2 is controlled by various regulons, depending on the physiological state of the culture.

In exponential-phase cultures, the antiperoxide OxyR regulon is primarily active [2], whereas during the transition to the stationary phase and starvation, the defense mechanisms are controlled by the alternative σ^S subunit of RNA polymerase within the large RpoS regulon [3]. In both cases, the regulation of the adaptive mechanisms occurs primarily at the level of transcription. The activity of promoters is regulated by transcription-regulating factors and the DNA topological state, which is related to the environmental conditions [4]. The level of DNA supercoiling is controlled by the energy-dependent topoisomerase system, whose activity is determined by the energy state of the cell [5, 6] and some other factors, including DNA-binding proteins (IHF, FIS, H-NS, HU) [3] and such topologically active cellular metabolites as polyamines, whose content changes considerably under stress [7]. In the present work, we studied the dependence of DNA topology in starving and growing *E. coli* cells subjected to hydrogen peroxide-induced oxidative stress on the cellular content of putrescine and the activity of its transport, as well as on the energy state of the cells.

MATERIALS AND METHODS

This work used *E. coli* K-12 (pBr 322). Cells were grown in M-9 synthetic medium under aerobic conditions in a 1-l ANKUM fermentor at a temperature of 37°C and controlled pH (pH 7.0). Glucose was added in portions (1–2 g/l) until the concentration of dry cell biomass (DCB) reached 2.5–3 g/l. The starving culture was subjected to oxidative stress either in the fermentor or in a temperature-controlled cell (75 ml) with cultivation parameters similar to those in the fermentor. Oxidative stress was imposed by the addition of 30% hydrogen peroxide to a final concentration of 3–60 mM.

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During cultivation, the content of polyamines and adenyl nucleotides was determined in sampled cells and medium by fluorimetric methods [8]. To estimate the total intracellular content of polyamines, cells were extracted with 0.4 N $HClO_4$, and to determine the soluble pool, they were extracted with 7.5% butanol. The cell adenylate charge was calculated by Atkinson's formula EC = (ATP + 0.5ADP/ATP + ADP + AMP) [9]. The concentration of potassium ions was measured on a PHLAFO var (Carl Zeiss) flame photometer. The degree of DNA supercoiling was studied by the plasmid method [7]. Cell biomass was calculated from the optical density (540 nm) using a calibration curve. The rate of cell death as dependent on the concentration of hydrogen peroxide was determined from viable cell counts determined by culture plating onto LB agar before and 15 min after the addition of hydrogen peroxide. Each experiment was run 3–7 times. Average data from replicate experiments are shown in the figures plotted using Statistica for Windows 5.0 software (StatsGraph regime). Vertical lines indicate the mean error values.

RESULTS AND DISCUSSION

Low concentrations of hydrogen peroxide (3–6 mM) caused the most pronounced cell damage in glucose-deficient *E. coli* cultures (Fig. 1). This type of cell death is a result of DNA damage caused by superoxide radicals or free hydroxyl radicals generated by one-electron H_2O_2 reduction with divalent iron ions [10]. Unlike exponentially growing cells [11], starving cells were resistant to higher peroxide concentrations (Fig. 1) and remained viable for 15 min in the presence of 60 mM H_2O_2. This seems to be the result of cell preadaptation to oxidative stress: during starvation for the carbon and energy source, the RpoS regulon is induced [3]. This regulon controls many genes, including those encoding the enzymes required for cell adaptation to oxidative stress in starving and stationary-phase cultures. These enzymes differ from those operating in exponentially growing *E. coli* cells; the most important of them are hydroperoxidase HPII (*katE*), which decomposes H_2O_2 to water and oxygen, and exonuclease III (*xthA*), which is involved in the repair of oxidatively damaged DNA.

DNA strand breaks caused by a low concentration of hydrogen peroxide lead to DNA relaxation in the first phase of the oxidative stress, as we demonstrated in experiments with exponentially growing *E. coli* cultures [11]. Oxidative stress in starving *E. coli* cultures led to similar changes in DNA topology (Fig. 2).

Since putrescine (the major polyamine in *E. coli*) was shown to be topologically active [7] and to operate as a scavenger of free radicals [12], we studied its content in the cells and medium under oxidative stress (Fig. 3). During starvation, the total pool of putrescine increased permanently and the rate of this increase did not change significantly after the addition of H_2O_2, whereas the content of putrescine dissolved in the cytoplasm increased dramatically after the addition of H_2O_2 (Fig. 3), suggesting a release of bound putrescine from polyanionic structures, mostly from DNA [13]. This process coincided with DNA relaxation (Fig. 2) and seems to be related to the topological activity of putrescine [7].

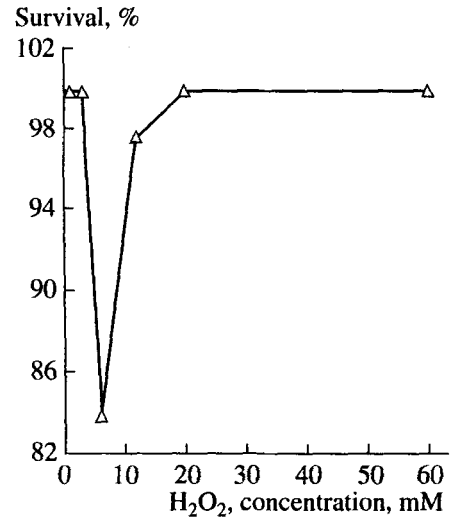

Fig. 1. Dependence of cell survival on the H_2O_2 concentration in a starving glucose-deficient *E. coli* culture.

The DNA relaxation due to its damage by the products of hydrogen peroxide decomposition and to putrescine dissociation was not compensated for by the increase in the cell adenylate charge that occurs in response to the addition of H_2O_2 (most probably, as a result of disturbed coupling between catabolism and anabolism), and that should stimulate the activity of the energy-dependent DNA gyrase (Fig. 4).

The initially low adenylate charge of the starving cells prior to the stress resulted in the low content of potassium in these cells and its permanent release into the medium due to the low activity of the energy-dependent transport system (Fig. 5). Against this background, the addition of H_2O_2 was accompanied by a short-term release of potassium from the cells, followed by the restoration of the intracellular potassium pool. In the potassium-limited exponential *E. coli* culture, the response to the addition of H_2O_2 was similar but much more pronounced (Fig. 6).

The relaxation period was followed by an increase in DNA supercoiling, most probably due to the activation of the repair systems by the low supercoiling level [14]. The restoration of prestress DNA topology evidently involved putrescine: a decrease in its free pool indicated its backward binding to DNA during this period (Fig. 3). The high energy state of the cells also stimulated the energy-dependent DNA gyrase activity [5, 6]. All these factors brought about a level of DNA supercoiling that exceeded the prestress level (Fig. 2).

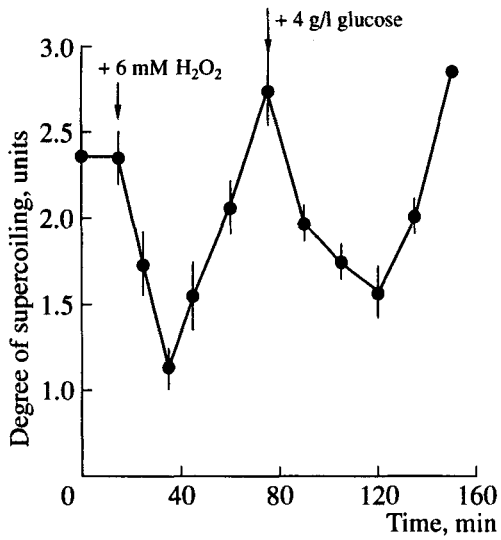

Fig. 2. Influence of oxidative stress on the DNA topology in a starving *E. coli* culture and after the addition of glucose.

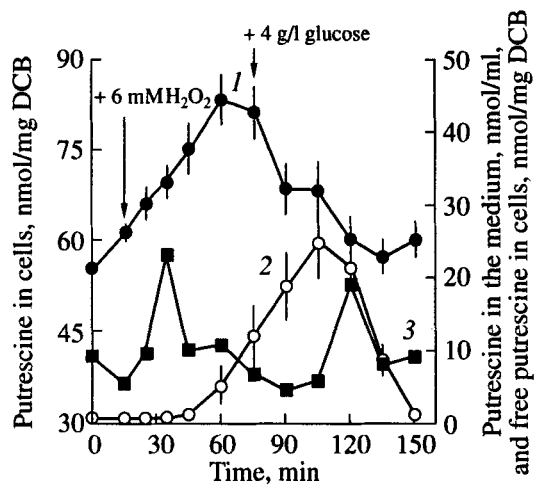

Fig. 3. Changes in the content of putrescine in cells and medium under oxidative stress in a starving *E. coli* culture and after the addition of glucose: (*1*) total putrescine concentration in cells; (2) putrescine concentration in the medium; (*3*) concentration of free putrescine in cells (extraction with butanol).

The putrescine release from the cells occurring concurrently with an increase in its intracellular pool (Fig. 3) suggests that the activity of the polyamine-synthesizing system was considerably stimulated in response to the oxidative stress, which is also characteristic of exponentially growing *E. coli* cultures [11]. An increased putrescine flow across the cytoplasmic membrane seems to protect membrane phospholipids against peroxide oxidation [15]. Due to the antioxidant activity of putrescine and the interaction of this diamine with the membrane, the integrity of the latter and the membrane energy functions are restored, as evidenced by an increase in the adenylate charge (Fig. 4) and the retention of potassium in the cells observed during this period.

After glucose was added to the starving *E. coli* culture in the presence of hydrogen peroxide, the described cycle of the topological changes was repeated (Fig. 2). The addition of glucose alone was not sufficient to induce this effect: in the absence of hydrogen peroxide, growth initiation is accompanied by an increase in DNA supercoiling, whereas the relaxation phase characteristic of the oxidative stress is omitted [16]. Provided that DNA topology correlates with the degree of cell adaptation to stress, the repeated cycle of the topological changes may be considered a manifestation of the second stress, which may be caused by the influx of additional reducing equivalents for Fenton's reaction [10]. On the other hand, the reason for the second stress may be lack of enzyme synthesis occurring in growing cells, in which the OxyR regulon, controlling hydroperoxidase I (*katG*), alkyl hydroperoxidase (*ahpCF*), glutathione oxidoreductase (*gor*), and other enzymes, is responsible for the cell adaptive reactions [2].

The DNA topology is known to influence the promoter accessibility and binding of RNA polymerase and transcription factors to DNA; this ultimately leads to the selective synthesis of adaptive stress proteins [4]. Fine adjustment of the adaptive systems seems to involve topologically active compounds, including polyamines, nucleotides that determine the energy state of the cell, and potassium ions, whose level fluctuates considerably under stress conditions.

After a short-term increase following the addition of glucose, the cell adenylate charge decreased again (Fig. 4) as a result of energy consumption caused by resumed growth under conditions of peroxide inhibition.

The content of cellular potassium sharply increased after the addition of glucose (Fig. 5), which was likely

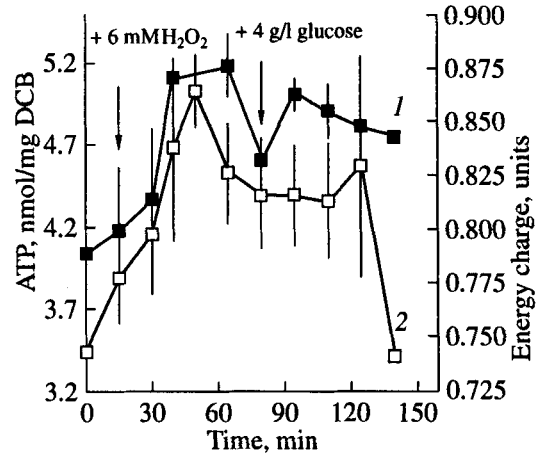

Fig. 4. Effect of H_2O_2 on (*1*) adenylate charge and (2) ATP content in *E. coli* cells during glucose starvation and growth initiation.

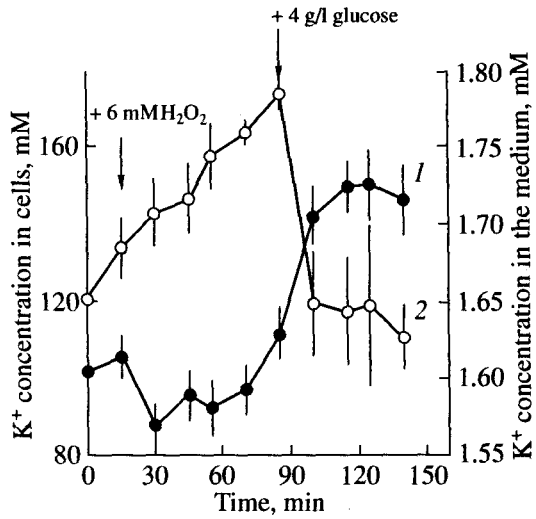

Fig. 5. Changes in the potassium concentration in (*1*) cells and (2) medium under oxidative stress in a starving *E. coli* culture and after the addition of glucose.

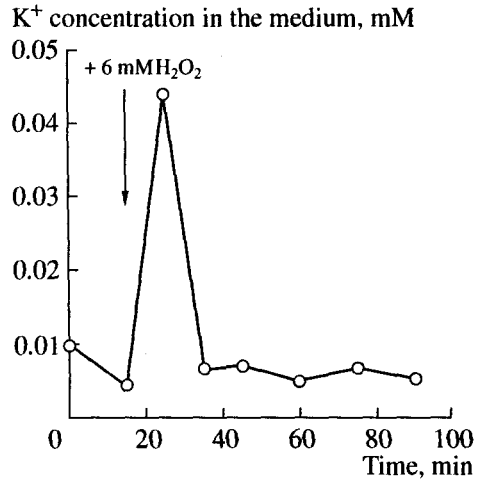

Fig. 6. Influence of oxidative stress on the content of extracellular potassium in a potassium-limited exponential *E. coli* culture.

caused by the activation of transport processes. The influx of potassium into cells was accompanied by a release of putrescine into the medium (Fig. 3). The role of the putrescine–potassium antiporter in this process cannot be excluded [17]. The temporary release of putrescine induced by the addition of glucose was followed by putrescine consumption due to the binding of this diamine to the cellular components, primarily DNA (Fig. 3). Restoration of the cellular potassium content and putrescine binding promoted the restoration of the prestress level of DNA supercoiling (Fig. 2).

Thus, the addition of the carbon and energy source to a starving *E. coli* culture in the presence of hydrogen peroxide leads to a second stress reaction of the cells. This fact supports the idea that, in exponentially growing and starving *E. coli* cultures, different systems are involved in cell defense against oxidative stress. Putrescine binding to DNA and its dissociation seems to modulate the DNA topological state, which regulates the expression of the adaptive genes. An increase in the activity of the polyamine-synthesizing system in response to oxidative stress results in a flow of putrescine across the cytoplasmic membrane; putrescine antioxidant activity protects membrane phospholipids and promotes restoration of the energy-generating cell functions.

REFERENCES

1. Demple, B., Redox Signaling and Gene Control in the *Escherichia coli* soxRS Oxidative Stress Regulon: A Review, *Gene*, 1996, vol. 179, pp. 53–57.
2. Gonzalez Flecha, B. and Demple, B., Transcriptional Regulation of the *Escherichia coli oxyR* Gene as a Function of Cell Growth, *J. Bacteriol.*, 1997, vol. 179, pp. 6181–6186.
3. Eisenstark, A., Calcutt, M.S., Becker Hapak, M., and Ivanova, A., Role of *Escherichia coli rpoS* and Associated Genes in Defence Against Oxidative Damage, *Free Radical Biol. Med.*, 1996, vol. 21, no. 7, pp. 975–983.
4. Wang, J.Y. and Syvanen, M., DNA Twist as a Transcriptional Sensor for Environmental Changes, *Mol. Microbiol.*, 1992, vol. 6, no. 14, pp. 1861–1866.
5. Mencel, R. and Gellert, M., Regulation of Genes for *Escherichia coli* DNA Girase: Homeostatic Control of DNA Supercoiling, *Cell*, 1983, vol. 34, pp. 105–113.
6. van Workum, M., van Dooren, S.J., Oldenburg, N., Molenaar, D., Jensen, P.R., Snoep, J.L., and Westerhoff, H.V., DNA Supercoiling Depends on the Phosphorylation Potential in *Escherichia coli, Mol. Microbiol.*, 1996, vol. 20, no. 2, pp. 351–360.
7. Tkachenko, A.G., Salakhetdinova, O.Ya., and Pshenichnov, M.R., Putrescine/Potassium Exchange as an Adaptive Response of *Escherichia coli* to Hyperosmotic Stress, *Mikrobiologiya*, 1997, vol. 66, no. 3, pp. 329–334.
8. Tkachenko, A.G. and Chudinov, A.A., Exchange of Polyamines between the Cell and the Medium as One of the Factors Determining the Development of Fed-Batch Cultures of *Escherichia coli, Mikrobiologiya*, 1989, vol. 58, no. 4, pp. 584–590.
9. Atkinson, D.E., Adenylate Energy Charge Is a Key Factor, *Trends Biochem. Sci.*, 1977, vol. 2, pp. 198–200.
10. Imlay, J.A. and Linn, S., DNA Damage and Oxygen Radical Toxicity, *Science*, 1988, vol. 240, pp. 1302–1309.
11. Tkachenko, A.G., Pshenichnov, M.R., Salakhetdinova, O.Ya., and Nesterova, L.Yu., The Role of Putrescine and the Energy State of *Escherichia coli* in the Regulation of DNA Topology during the Adaptation to Oxidative Stress, *Mikrobiologiya*, 1999, vol. 68, no. 1, pp. 27–32.
12. Ha, H.C., Sirisoma, N.S., Kuppusamy, P., Zweier, J.L., Woster, P.M., and Casero, R.A., The Natural Polyamine Spermine Functions Directly as a Free Radical Scaven-

ger, *Proc. Natl. Acad. Sci. USA*, 1998, vol. 95, pp. 11140–11145.

13. Kashiwagi, K., Kobayashi, H., and Igarashi, K., Apparently Unidirectional Polyamine Transport by the Proton Motive Force in Polyamine-Deficient *Escherichia coli*, *J. Bacteriol.*, 1986, vol. 165, pp. 972–977.
14. Urios, A., Herrera, G., Aleixandre, V., and Blanco, M., Expression of *recA* Gene Is Reduced in *Escherichia coli* Topoisomerase I Mutants, *Mutation Res.*, 1990, vol. 243, pp. 267–272.
15. Tadolini, B., Polyamine Inhibition of Lipoperoxidation, *Biochem. J.*, 1988, vol. 249, pp. 33–36.
16. Balke, V.L. and Gralla, G.D., Changes in the Linking Number of Supercoiled DNA Accompany Growth Transitions in *Escherichia coli*, *J. Bacteriol.*, 1987, vol. 169, pp. 4499–4506.
17. Tkachenko, A.G. and Chudinov, A.A., Participation of Putrescine in the Antiport Mechanism of Potassium Transport in *Escherichia coli*, *Curr. Microbiol.*, 1994, vol. 28, pp. 81–83.