J. Microbiol. Biotechnol. (2004), **14**(3), 466–469

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Adaptive Responses of *Escherichia coli* for Oxidative and Protein Damage Using Bioluminescence Reporters

MIN, JIHO AND MAN BOCK GU*

National Research Laboratory on Environmental Biotechnology, Kwangju Institute of Science and Technology (K-JIST), 1 Oryong-dong, Puk-gu, Gwangju 500-712, Korea

Received: April 19, 2003
Accepted: December 17, 2003

Abstract The recombinant bioluminescent *Escherichia coli* strains, DPD2511 and TV1061 containing the *katG* and *grpE* promoters, respectively, from *Vibrio fischeri* fused to *luxCDABE*, were used to detect the adaptive and repair responses to oxidative damage caused by hydrogen peroxide (H_2O_2), and protein damage due to phenol. The response ratio, represented as the bioluminescence induced in subsequent inductions of DPD2511 and TV1061 with the mother cells previously induced by each chemical, i.e., H_2O_2 and phenol during the previous induction stage, decreased suddenly compared with the ratio of the control culture of each strain, meaning there is a possible adaptive response to stress caused by chemicals. Protein damage due to phenol was completely repaired by the second culturing after the initial induction, as was oxidative damage caused by H_2O_2, which was also rapidly repaired, as detected by the recovery of bioluminescence level. This result suggests that *E. coli* promptly adapt and repair oxidative and protein damage by H_2O_2 and phenol completely.

Key words: Stress adaptation, *katG* promoter, *grpE* promoter, oxidative damage, protein damage

Living cells show a rapid cellular response when they are exposed to adverse environmental conditions. This ubiquitous phenomenon is commonly designated as a stress response, and it can be considered as a general cellular reaction to metabolic disturbances [14, 16]. Every organism has elaborate mechanisms to cope with various types of stresses induced by toxic chemicals inside the cell [4, 6, 10]. Exposure of *Escherichia coli* to mutagens that damage DNA or interfere with DNA replication results in the induction of a diverse set of physiological responses, termed the SOS response, controlled under the SOS regulon [20]. In addition, the OxyR regulon controls expression of many proteins, such as catalase, alkyl hydroperoxide reductase, and glutathione, to reduce and repair cellular components damaged by oxidative stress [6], and the heat-shock regulon responds to molecular events such as protein damage, operating upon a shift in temperature or chemical exposure [16]. Therefore, the responses of each regulon to these stresses mean there is an adaptive response to each stress and it could be used to study and evaluate the effectiveness of each regulon system. In this study, a recombinant bioluminescent *Escherichia coli*, DPD2511 with the *katG* promoter fused to *luxCDABE* originated from *Vibrio fischeri* [1, 9, 15] and TV1061 with the *grpE* promoter fused to *luxCDABE* [5, 15, 21], were used to observe adaptive responses for oxidative damage and protein damage, respectively, after the cellular components were damaged by two different toxic chemicals, namely, an oxidative damaging agent [hydrogen peroxide (H_2O_2)], and a protein damaging agent (phenol).

Materials and Methods

Stain, Growth, and Media

The recombinant bacterial strains *E. coli* DPD2511 and TV1061, containing a *katG::luxCDABE* and *grpE::luxCDABE* fusion, respectively, were used in this study [1, 5, 9, 15, 21]. Cells were grown in Luria-Bertani (LB) medium (Difco-BRL, Gaithersburg, MD, U.S.A.) containing 25 mg/l of kanamycin monosulfate (Sigma Co., St. Louis, MO, U.S.A.) adjusted at pH 7.0, 30°C, with shaking at 250 rpm. A flask containing 100 ml of sterile medium was inoculated with 2 ml of inoculum overnight. When the optical density at 600 nm (OD_{600}) reached 0.08, chemicals were added to the culture media [2, 3]. The bioluminescence (arbitrary units, AU) emitted was monitored using a highly sensitive luminometer (Model 20e, Turner Design, CA, U.S.A.) and the cell growth was measured by UV/Vis spectrophotometer (Perkin Elmer Co., U.S.A.) at 600 nm. All bioluminescence

*Corresponding author
Phone: 82-62-970-2440; Fax: 82-62-970-2434;
E-mail: mbgu@kjist.ac.kr

(BL) values were represented by specific bioluminescence (SBL), defined as the volumetric light emission (arbitrary unit, AU) divided by the optical density of the cell suspension (OD_{600}). The maximum relative SBL ratio here is defined as the ratio of the SBL of the induced cells divided by SBL of the uninduced cells.

Adaptation of *E. coli* to Oxidative and Protein Damage Through a Serial Passage of the Induced Cells

When OD_{600} of the cell suspension (100 ml) reached 0.8 by induction with 100 ppb (mg/l) of H_2O_2 for DPD2511, and 400 ppm of phenol for TV1061 (Sigma Co.), the cell suspension was harvested by centrifugation (Hanil Co., Inchon, Korea) for 30 min at 2,600 ×*g* and 4°C to remove the chemicals. The concentration of each chemical used in this study was confirmed to be sub-lethal, *i.e.*, without growth inhibition based upon a previous study [10, 19]. The supernatant was discarded and the pellet was resuspended in 100 ml of LB medium. A new flask with 100 ml of LB medium was inoculated with 2 ml of the resulting suspension. When the optical density reached 0.08, the same chemical at the same concentration was added to the culture media again for the second passage. When the OD_{600} of the cells reached 0.8, the procedure was repeated for the second time. Experiments to determine the adaptive and repair responses that occur after DNA damage were conducted with a serial dilution protocol, *i.e.*, the induced cells from the first passage were transferred to two separate flasks, one for a control and the other for the second induction in the next passage. In other words, the induction by the same concentration of the chemical was performed in the second flask at each stage. Using the control cells as the seed for the next passage allowed us to monitor the adaptation of the cells after oxidative and protein damage by the induction of the exposed culture to that of the control. This procedure was repeated for a total of seven times as shown in Fig. 1. During all of these experiments, the bioluminescence was monitored and recorded. All bioluminescence (BL) values are presented as the specific bioluminescence (SBL), defined as light emission (arbitrary unit, AU), divided by the optical density of the cell suspension (OD_{600}). The relative SBL (RSBL) ratio is defined as the ratio of the maximum SBL of the challenged (or 'induced') cells divided by maximum SBL of the 'uninduced' cells. The percentage of inducibility at each passage was used to explain the adaptive responses of the cells to the DNA damage caused by each chemical tested in this study, and is defined as the ratio of the RSBL of the initially exposed culture to those at each passage.

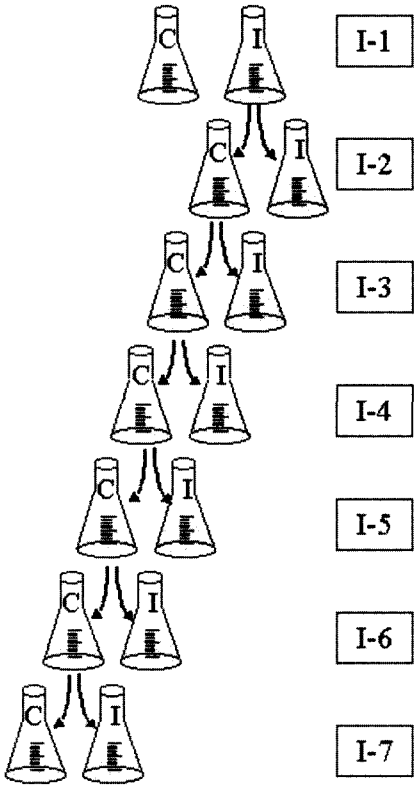

Fig. 1. Experimental scheme to test adaptive and repair responses by oxidative and protein damage.
"C" is the control, *i.e.*, uninduced strain, and "I" is the induced strain. DPD2511 and TV1061 were exposed to H_2O_2 and phenol, respectively. In order to determine how much time is required to recover their original sensitivity after one induction, it was tested to the seventh stage.

Error Analysis

All the experiments were performed in triplicate for statistical analysis. Three data points were used to calculate standard deviation for the error bars.

Results and Discussion

In this study, some adaptive responses to the oxidative and protein stresses caused by two different toxic chemicals, H_2O_2 and phenol, were studied and compared using two recombinant *E. coli* strains, DPD2511 and TV1061, with the *katG* and *grpE* promoters fused with the *luxCDABE* operon, respectively.

H_2O_2, at a concentration of 100 ppb that was previously observed to be sub-lethal [10, 19], was used to analyze the adaptive responses for oxidative damage in DPD2511. The experiments to determine the adaptive and repair responses that occur after oxidative damage were conducted using serial culture passages, *i.e.*, the induced cells from the first passage were transferred to two separate flasks, one for using as a control and the other for the second induction with the same chemical at the same concentration (Fig. 1). The results are shown in Fig. 2. When subcultured from the originally induced flask, it was found that the inducibility of the cells to H_2O_2 in the second passage was 43%,

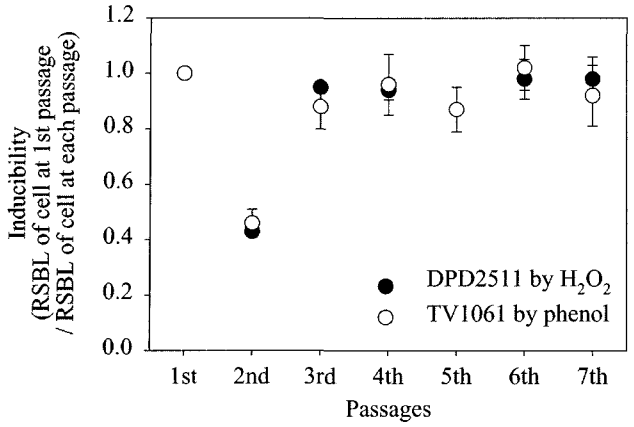

Fig. 2. Inducibility comparing relative specific biloluminescence (RSBL) of the initially induced cell, using the RSBL values from the induced cell at each passage that were exposed to H_2O_2 and phenol and were then serially subcultured and further induced. ● is the response of DPD2511 with 100 ppb H_2O_2; ○ is the response of TV1061 with 400 ppb phenol.

indicating that the recombinant *E. coli* had adapted and was resistant to further insults by H_2O_2 (Fig. 2). This result was consistant with that of the adaptive response for protein damage within TV1061 caused by phenol. When subcultured from the originally induced flask, it was shown that the inducibility of the TV1061 to 400 ppb phenol in the second passage was 46%, and it is also indicative of the fact that the recombinant *E. coli* had adapted and was resistant to further exposure to phenol (Fig. 2).

In a previous study, when the recombinant *E. coli* strain DPD2794 with the *recA* promoter fused to the *luxCDABE* was exposed to mitomycin C (MMC), the inducibility was approximately half the original percentage for the third to fifth passages and then began to increase again from the sixth [17]. The bioluminescence ratio of DPD2794 recovered to a normal response ratio by the seventh passage, showing that the induced cells could recover their sensitivity [17]. However, DPD2511 and TV1061 showed different patterns of adaptation when compared with that of DPD2794. It was found that this adaptation due to H_2O_2 and phenol addition was lost if the cells were not induced in the stage just prior to those additions, indicating that the damage caused by these two chemicals is promptly repaired and that the proteins produced to repair the damage may be turned over more quickly than when the cells are damaged by MMC.

Adaptive and repair responses brought on by oxidative stress caused by hydrogen peroxide include the OxyR-dependent regulation of numerous genes [6]. Reactive oxygen species (ROS) cause oxidative stress in bacteria, and to adapt and repair the oxidative damage by oxidative stress, bacteria produce several proteins including catalase, alkyl hydroperoxide reductase, and glutathione, which have been shown to be under the control of a transcriptional activator, OxyR [6, 8]. In addition, adaptive and repair responses for protein damage caused by phenol are controlled by the heat-shock regulon [16]. The level of heat-shock proteins needed to respond to protein damage depends on the amount of σ^{32} produced. The major heat-shock proteins, DnaK, DnaJ, and GrpE, bind to σ^{32} under protein-damaging conditions and then act as a chaperone to adapt and repair protein damage [16]. Therefore, *E. coli* is capable of rapidly adapting to H_2O_2 and phenol-induced oxidative and protein damage. Along with the results presented here, it could be suggested that *E. coli* promptly adapts and completely repairs the oxidative and protein damage caused by H_2O_2 and phenol, as compared with DNA repair by MMC, which takes more time to repair.

Acknowledgments

This work was supported by the National Research Laboratory (2001 NRL) program of the Korea Institute of Science and the Technology Evaluation and Planning (Project No. M10104000094-01J000004100). We would like to express our gratitude for this support.

References

1. Belkin, S., D. R. Smulski, A. C. Vollmer, T. K. Van dyk, and R. A. LaRossa. 1996. Oxidative stress detection with *Escherichia coli* harboring a *katG::lux* fusion. *Appl. Environ. Microbiol.* **62:** 2252-2256.
2. Britz, M. L., N. Simonov, and U.-H. Chun. 1997. Stabilization of bioluminescence of immobilized *Photobacterium phosphoreum* and monitoring of environmental pollutants. *J. Microbiol. Biotechnol.* **7:** 242-249.
3. Britz, M. L., N. Simonov, and U.-H. Chun. 1997. Immobilized luminescent cell-based flow through monitoring of environmental pollutants. *J. Microbiol. Biotechnol.* **7:** 250-257.
4. Bromberg, R., S. M. George, and M.W. Peck. 1998. Oxygen sensitivity of heated cells of *Escherichia coli* O157:H7. *J. Appl. Microbiol.* **85:** 231-237.
5. Choi, S. H. and M. B. Gu. 2001. Phenolic toxicity - detection and classification through the use of recombinant bioluminescent *Escherichia coli* cells. *Environ. Toxicol. Chem.* **20:** 248-255.
6. Demple, B. 1991. Regulation of bacterial oxidative stress genes. *Annu. Rev. Gen.* **25:** 315-337.
7. Deng, Y., J.-H. Ryu, and L. R. Beuchat. 1999. Tolerance of acid-adapted and non-adapted *Escherichia coli* O157:H7 cells to reduced pH as affected by type of acidulant. *J. Appl. Microbiol.* **86:** 203-210.
8. Dukan, S. and D. Touati. 1996. Hypochlorous acid stress in *Escherichia coli*: Resistance, DNA damage, and comparison with hydrogen peroxide stress. *J. Bacteriol.* **178:** 6145-6150.

9. Gu, M. B. and S. H. Choi. 2001. Monitoring and classification of toxicity using recombinant bioluminescent bacteria. *Wat. Sci. Tech.* **43:** 147-154.
10. Gu, M. B., J. Min, and R. A. LaRossa. 2000. Bacterial bioluminescent emission from recombinant *Escherichia coli* harboring a *recA::luxCDABE* fusion. *J. Biochem. Biophys. Methods* **45:** 45-56.
11. Huh, N.-E., N.-S. Choi, Y.-K. Seo, T.-S. Yu, and H.-S. Lee. 1994. Characterization of a cadmium-resistant yeast strain in response to cadmium or heat shock stress. *J. Microbiol. Biotechnol.* **4:** 30-35.
12. Juneja, V. K., P. C. Klein, and B. S. Marmer. 1998. Heat shock and thermotolerance of *Escherichia coli* O157:H7 in a model beef gravy system and ground beef. *J. Appl. Microbiol.* **84:** 677-684.
13. Kwak, Y. H., J. K. Sung, Y. L. Ki, and K. B. Han. 2000. Stress responses of the *Escherichia coli groE* promoter. *J. Microbiol. Biotechnol.* **10:** 63-68.
14. Lee, H. J. and M. B. Gu. 2003. Effects of benzo[a]pyrene on genes related to the cell cycle and cytochrome P450 of *Saccharomyces cerevisiae. J. Microbiol. Biotechnol.* **13:** 624-627.
15. Lee, H. W., S. H. Choi, and M. B. Gu. 2003. Response of bioluminescent bacteria to sixteen azo dyes. *Biotech. Bioprocess. Eng.* **8:** 101-105.
16. Mager, W. H. and A. J. J. Kruijff. 1995. Stress-induced transcriptional activation. *Microbiol. Rev.* **59:** 506-531.
17. Min, J. and M. B. Gu. 2003. Acclimation and repair of DNA damage in *Escherichia coli. Submitted to Journal of Applied Microbiology. Accepted for publication.*
18. Min, J. and M. B. Gu. 2003. Genotoxicity assay using a chromosomally-integrated bacterial *recA* promoter::*lux* fusion. *J. Microbiol. Biotechnol.* **13:** 99-103.
19. Min, J., E. J. Kim, R. A. LaRossa, and M. B. Gu. 1999. Distinct responses of a *recA::luxCDABE Escherichia coli* strain to direct and indirect DNA damaging agents. *Mutat. Res.* **442:** 61-68.
20. Rupp, W. D. 1996. The SOS response of *Escherichia coli*, pp. 1400-1416. *In* Frederick, C. N. *et al.* 2nd eds. *Escherichia coli and Salmonella: Cellular and Molecular Biology.* American Society for Microbiology Press. Washinton, DC, U.S.A.
21. Van Dyk, T. K., D. R. Smulski, T. R. Reed, S. Belkin, A. C. Vollmer, and R. A. LaRossa. 1995. Responses to toxicants of an *Escherichia coli* strain carrying a *uspA::lux* genetic fusion and an *E. coli* strain carrying a *grpE::lux* fusion are similar. *Appl. Environ. Microbiol.* **61:** 4124-4127.