

## **Inhibition of UV-induced SOS responses by 3-Aminobenzamide in *Escherichia coli***

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### ABSTRACT

The effects of 3-aminobenzamide (3 ABA), an inhibitor of poly(ADP-ribose) synthetase, on UV-induced SOS responses were studied in *Escherichia coli*. The lethal effect of UV irradiation was enhanced by 3 ABA in an excision repair defective strain (*uvrA*) and in the parental wild-type strain. In contrast, in a recombination defective mutant (*recA*), 3 ABA inhibited UV-caused cell mortality. An apparent depression of UV-induced mutation by 3 ABA was observed in *uvrA*. In addition, induction of the *umu*<sup>+</sup> gene expression by UV was depressed by 3 ABA in both *uvrA* and wild-type strains. Prophage induction from  $\lambda$ -lysogenized cells by UV was also depressed by 3 ABA. Taken together, these results demonstrate that 3 ABA inhibits a diverse set of SOS responses induced by UV irradiation.

**Key Words:** Poly(ADP-ribose), 3-aminobenzamide, SOS responses, *E. coli*, mutation, prophage induction, *umu*<sup>+</sup> gene.

### 1. INTRODUCTION

In recent years, there have been several published reports on the role of poly(ADP-ribose) synthesis in DNA repair. In these papers, it has been shown that following treatment of eukaryotic cells with DNA damaging agents, a drastic change occurs in the concentration of NAD<sup>+</sup>, a substrate of poly(ADP-ribose) synthetase (Berger *et al.* 1979, Skidmore *et al.* 1979). When inhibitors of poly(ADP-ribose) synthetase such as 3 ABA are present during DNA repair, depression of NAD<sup>+</sup> content and enhanced cell mortality are noted (Durkacz *et al.* 1980). Thus, polyADP-ribosylation is currently believed to play a significant role in the DNA repair processes, particularly during late stages of ligation.

In prokaryotic bacterial cells, DNA damaging agents induce a diverse set of cellular "SOS" responses (Radman 1975, Witkin 1976). These consist of the following: (1) activation of a special DNA repair system, (2) induction of mutations, (3) synthesis of certain proteins, particularly *recA* and *umuC* proteins, at high levels, (4) proteolytic cleavage of repressors of certain pro-

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*Abbreviations:* 3 ABA, 3-aminobenzamide; UV, ultraviolet light; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; *E. coli*, *Escherichia coli*.

phages such as  $\lambda$ , followed by expression of phage genes (Gudas and Pardee 1976, McEntee *et al.* 1976). In *E. coli*, the SOS responses are observed in the *uvrA* and wild-type strains but not in the *recA* strain. There have been no published reports on the importance of polyADP-ribosylation in DNA repair processes and the poly(ADP-ribose) synthetase in *E. coli*. In this paper, we will report on the inhibition of UV-induced SOS responses by 3 ABA, one of the specific inhibitors of poly(ADP-ribose) synthetase.

## 2. MATERIALS AND METHODS

*Strains.* For lethality and mutation tests, the three strains of *Escherichia coli* used here were H/r 30 R (*argF<sub>amber</sub>*), Hs 30 R (*argF<sub>amber</sub> uvrA*) and NG 30 (*argF<sub>amber</sub> recA*). Two strains of  $\lambda$ -lysogenized *E. coli* W3110 ( $\lambda^+$ ) and W3110 ( $\lambda ind^+$ ) were used for the prophage induction tests. To measure the plaque forming ability of the induced phage, *E. coli* K 12-103 was used as an indicator strain. For induction tests of the *umu*<sup>+</sup> gene expression, four strains of *E. coli*, KY 700 (wild-type for repair), KY 703 (*lexA*), KY 705 (*recA*) and KY 706 (*uvrA*), were used. These cells were transformed with plasmid pSK 1002, a derivative of pMC 1403 (Casadaban *et al.* 1980). Plasmid pSK 1002 carries the promoter of the *umu*<sup>+</sup> operator, and the *umuD*<sup>+</sup> and *umuC'*-*lacZ* fusion genes (Shinagawa *et al.* 1983). Therefore, the UV induced activity of the *umu*<sup>+</sup> gene expression was determined by measuring the enzymatic activity of  $\beta$ -galactosidase, since *lacZ*<sup>+</sup> gene expression is controlled by the *umu*<sup>+</sup> operator. The remaining genetic characteristics of all strains used here have been described in detail elsewhere (Ohnishi *et al.* 1985, Sato *et al.* 1985).

*Media and chemicals.* For bacterial growth, Luria (L)-medium was used. Davis minimal medium was used for mutation tests.  $\lambda$ -dilution buffer and  $\lambda$ -broth were used for prophage induction tests. 3 ABA, produced by Tokyo Kasei Kogyo Co. (Tokyo, Japan), was dissolved in dimethylsulfoxide at the indicated concentrations. The contents of all media used here have been described previously (Ohnishi *et al.* 1977, Sato *et al.* 1985).

*UV-irradiation.* *E. coli* cells in the growth phase were suspended in saline (0.9% NaCl) at a concentration of approximately  $3 \times 10^8$  cells/ml after washing twice with saline. The cells were irradiated with a germicidal lamp (254 nm), the dose rate of which was 1 J/m<sup>2</sup>/sec. All experiments were carried out under yellow light to prevent photoreactivation.

*UV-lethality.* After UV-irradiation, the cells were diluted with saline. A fixed volume of cell suspension was placed on bouillon agar with and without 30 mM 3 ABA. This concentration of 3 ABA is known to have almost no effect on the colony forming ability of *E. coli* cells. The number of colonies was

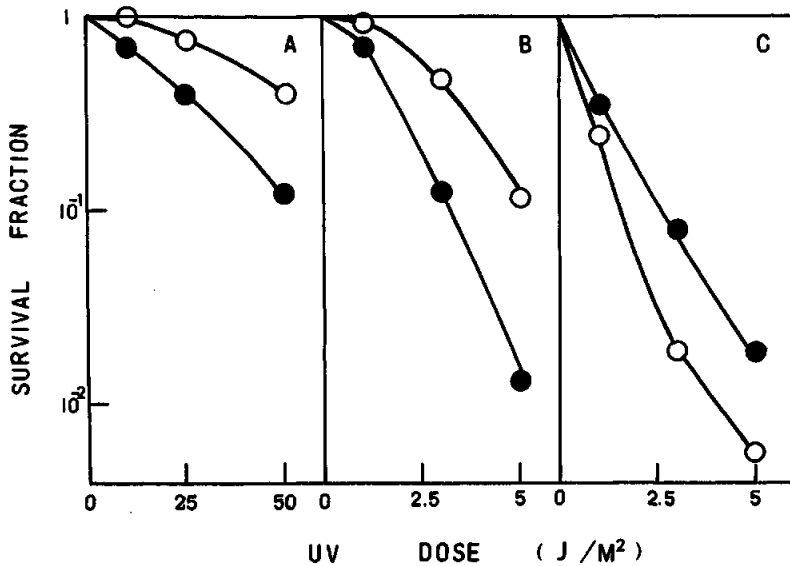


Fig. 1. Effect of 3 ABA on UV-lethality.

A, H/r 30 R (wild-type for repair); B, Hs 30 R (*uvrA*); C, NG 30 (*recA*). ○, without 3 ABA; ●, with 3 ABA.

counted after incubation for 20 hours at 37 °C.

*Induced mutation.* After UV-irradiation, the cells were washed and re-suspended in Davis minimal medium containing 20 µg/ml arginine with and without 30 mM 3 ABA. After incubating for 2 hours at 37°C, the cells were washed and spread on agar containing Davis minimal medium without arginine. The number of revertants was counted after 48 hours incubation at 37°C.

*Induction of *umu*<sup>+</sup> gene expression.* UV irradiation was applied to cells of the KY series at a dosage of 10 J/m<sup>2</sup>. The 3 × 10<sup>8</sup> cells per ml were then incubated with 3 ABA at various concentrations for 1 hour at 37°C. Subsequently, the cells were transferred to L-broth and incubated for 1 hour at 37°C to allow gene expression to take place. Measurements of cell concentration and β-galactosidase activity were then made. The other details of this procedure have been described elsewhere (Sato *et al.* 1985).

*Prophage induction.* UV irradiation was applied to W 3110 (*λ*<sup>+</sup>) and W 3110 (*λ ind*<sup>+</sup>) at dosages of 30 J/m<sup>2</sup> and 3 J/m<sup>2</sup>, respectively. The cells were then incubated with 3 ABA at various concentrations for 100 min at 37°C. The cell lysates were treated with chloroform and diluted with *λ*-dilution buffer. Following this, induced phages were spread on *λ*-broth agar with soft agar containing the indicator cells. After incubation for 20 hours at 37°C, the number of plaque forming units (PFU) was counted.

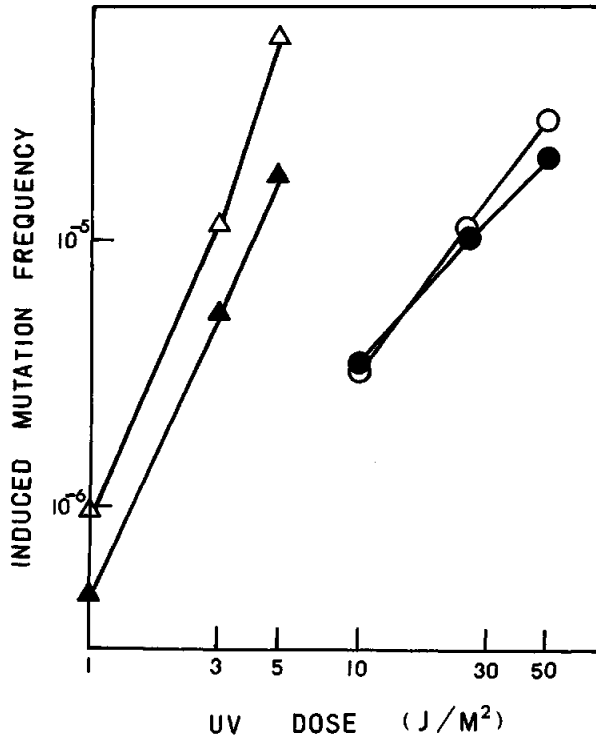


Fig. 2. Effect of 3ABA on UV-induced mutation. Circles, H/r 30 R (wild-type for repair); triangles, Hs 30 R (*uvrA*). Open symbols, without 3ABA; closed symbols, with 3ABA.

### 3. RESULTS AND DISCUSSION

Effects of 3ABA on UV dose-survival curves of H/r 30 R (wild-type for repair), Hs 30 R (*uvrA*) and NG 30 (*recA*) are shown in Fig. 1. The addition of 3ABA following UV irradiation enhanced cell killing in wild-type and *uvrA* strains. The sensitivity of each strain to UV as determined by its LD<sub>50</sub>, was increased by 200% with 3ABA. In contrast, the *recA* strain showed partial resistance to UV irradiation when 3ABA was present. These results indicate that 3ABA may inhibit certain DNA repair systems apart from excision repair.

Effects of 3ABA on induced mutation by UV irradiation are shown in Fig. 2. When 3ABA was added following UV-irradiation, the induced mutation frequency in *uvrA* was depressed to approximately 50% of the level without 3ABA. In the wild-type strain on the contrary, the depression of induced mutation by 3ABA was almost negligible. To determine whether the inhibition by 3ABA is regulated by *umuD*<sup>+</sup>*C*<sup>+</sup> genes, which play the crucial role in UV-induced mutations, we examined the effect of 3ABA on *umu*<sup>+</sup>

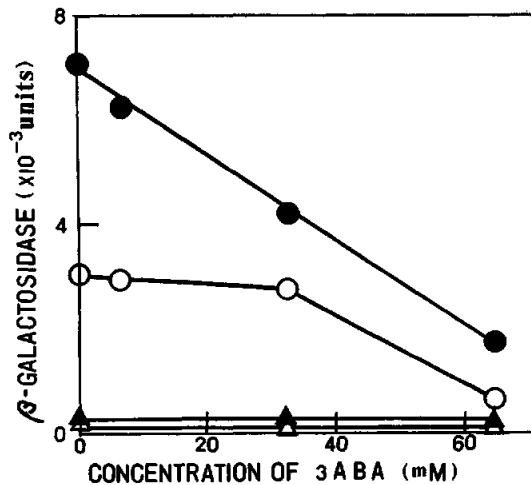


Fig. 3. Effect of 3 ABA on induction of the *umu*<sup>+</sup> gene expression.

Following UV irradiation, the cells were incubated for 2 h with 3 ABA at the indicated concentrations to induce the *umu*<sup>+</sup> gene expression. ○, KY 700 (wild-type for repair); ●, KY 706 (*uvrA*); ▲, KY 703 (*lexA*); △, KY 705 (*recA*).

gene expression (Fig. 3). Irradiation with UV could induce a high enzymatic activity of  $\beta$ -galactosidase, i. e., a high activity of *umu*<sup>+</sup> gene expression, in KY 706 (*uvrA*) and KY 700 (wild-type for repair). The induced activity in the *uvrA* strain was higher than that in the wild-type strain. In contrast, negligible induced activity was detected in the KY 703 (*lexA*) and the KY 705 (*recA*) strains. The presence of 3 ABA during the gene expression significantly depressed the induction of the *umu*<sup>+</sup> gene expression in *uvrA* and wild-type strains. These results lead us to suggest that 3 ABA depresses SOS-repair which contains error-prone processes.

Since UV irradiation induces  $\lambda$ -phages from  $\lambda$ -lysogenized cells, we examined the effect of 3 ABA on the prophage induction. The results are shown in Fig. 4. Although efficient induction was detected in W3110 ( $\lambda$ ind<sup>+</sup>) and W3110 ( $\lambda$ <sup>+</sup>), phage induction was markedly depressed by 3 ABA in both strains. These results support the earlier hypothesis that 3 ABA depresses at least one of the SOS responses.

In the present experiments, we have shown an apparent depression by 3 ABA of a diverse set of UV irradiation induced SOS responses. Previously we reported the depression of UV-induced mutations by 3 ABA in a low eukaryotic organism, *Dictyostelium discoideum* (Ohnishi *et al.* 1982). In the same organism, we also observed caffeine-effected repair inhibition of UV damaged DNA similar to that reported here for 3 ABA (Ohnishi *et al.* 1981). It is well known that caffeine inhibits the post-replication repair system which is thought to be one of the error prone repair processes (Kihlman 1977,

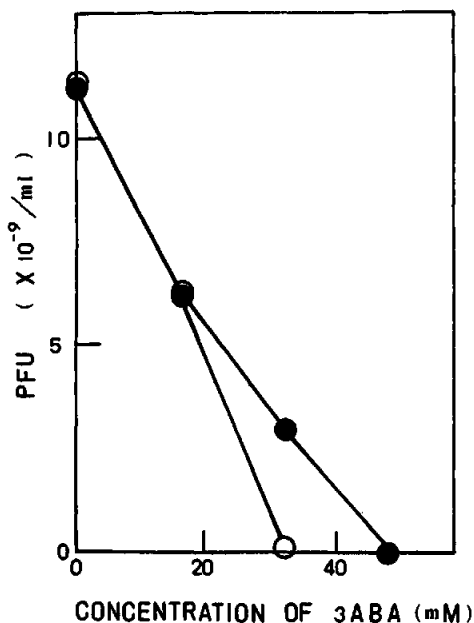


Fig. 4. Effect of 3 ABA on prophage induction.

Following UV irradiation, the cells were incubated for 100 minutes with 3 ABA at the indicated concentrations in order to induce prophage.  $\circ$ , W 3110 ( $\lambda^{ind+}$ );  $\bullet$ , W 3110 ( $\lambda^+$ ).

Timson 1977). Caffeine as well as 3 ABA is also well known to be one of the specific inhibitors of poly(ADP-ribose) synthetase. Therefore, poly(ADP-ribose) synthesis may have an important role in the SOS repair system of *E. coli*, although the presence of poly(ADP-ribose) synthetase in prokaryotic cells has not been documented as of this writing. However, it has been reported that *E. coli* cells contain histone-like proteins (Varshavsky *et al.* 1979, Hubscher *et al.* 1980) which could possibly be modified by poly(ADP-ribose) synthetase. The depression of UV-induced SOS responses by 3 ABA may be due to the inhibition of chromatid protein modification during the ligation stage in SOS repair systems in *Escherichia coli* as well as in eukaryotic cells (Durkacz *et al.* 1980, Ohashi *et al.* 1983). As another possibility, we may be unable to neglect that 3 ABA inhibits the expression of specific genes which relate to SOS responses.

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#### REFERENCES

- BERGER, N. A., SIKORSKI, G. W., PETZOLD, S. J. and KUROHARA, K. K. (1979) Association of poly(ADP-ribose) synthesis with DNA damage and repair in normal human lymphocytes. *J. Clin. Invest.* **63**, 1164-1171.

- CASADABAN, M. J., CHOU, J. and COHEN, S. N. (1980) In vivo gene fusions that join an enzymatically active  $\beta$ -galactosidase segment to amino-terminal fragments of exogenous proteins: *Escherichia coli* plasmid vectors for the deletion and cloning of translational signals. *J. Bacteriol.* **143**, 971-980.
- DURKACZ, B. W., NDUKZ, N., OMIJJI, O., SHALL, S. and ZIA'EE, A. A. (1980) ADP-ribose and DNA repair. (Eds. Smulson, M. E. and Sugimura, T.) In: *Novel ADP-ribosylations of Regulatory Enzymes and Proteins*. Elsevier-North Holland, Amsterdam, pp. 207-216.
- GUDAS, L. J., and PARDEE, A. B. (1976) DNA synthesis inhibition and induction of protein X in *Escherichia coli*. *J. Mol. Biol.* **101**, 459-478.
- HUBSCHER, U., LUTS, H. and KORNBERG, A. (1980) Novel histone H2A-like protein of *Escherichia coli*. *Proc. Natl. Acad. Sci. (USA)* **77**, 5097-5101.
- KIHLMAN, K. R. (1977) Caffeine and Chromosomes. Elsevier-North Holland, Amsterdam.
- MCENTEE, K., HESSE, J. E. and EPSTEIN, W. (1976) Identical and radiochemical purification of the *recA* protein of *Escherichia coli* K-12. *Proc. Natl. Acad. Sci. (USA)* **73**, 3979-3983.
- OHASHI, Y., UEDA, K., KAWAICHI, M. and HAYAISHI, O. (1983) Activation of DNA ligase by poly (ADP-ribose) in chromatin. *Proc. Natl. Acad. Sci. (USA)* **80**, 3604-3607.
- OHNISHI, T., EIMOTO, H. and OKAICHI, K. (1982) Enhancement of N-methyl-N'-nitro-N-nitrosoguanidine sensitivity of *Dictyostelium discoideum* by 3-aminobenzamide. *Photochem. Photobiol.* **35**, 515-519.
- OHNISHI, T., HARADA, Y., OHASHI, Y., KONISHI, Y. and NOZU, K. (1985) Induction of SOS responses in *Escherichia coli* by panfuran-S, 3-di(hydroxymethyl)-amino-6-(5-nitro-2-furyl-ethenyl)-1, 2, 4-triazine. *Mutation Res.* **146**, 243-247.
- OHNISHI, T., OKAICHI, K., OHASHI, Y. and NOZU, K. (1981) Effects of caffeine on DNA repair of UV-irradiated *Dictyostelium discoideum*. *Photochem. Photobiol.* **33**, 79-83.
- OHNISHI, T., YAMADA, S., NOMURA, N. and NOZU, K. (1977) Mutagenicity of antimicrobial medicine containing acetylfuratrizine. *Jpn. J. Genet.* **52**, 309-314.
- RADMAN, M. (1975) SOS repair hypothesis; phenomenology of an inducible DNA repair which is accompanied by mutagenesis. (Eds. Hanawalt, P. C. and Setlow, R. B.) In: *Molecular Mechanisms for Repair of DNA*. Plenum Press, New York pp. 355-367.
- SATO, N., OHNISHI, T., TANO, K., YAMAMOTO, K. and NOZU, K. (1985) Induction of *umuC*<sup>+</sup> gene expression in *Escherichia coli* irradiated by near ultraviolet light. *Photochem. Photobiol.* **42**, 135-139.
- SHINAGAWA, H., KATO, T., ISE, T., MAKINO, K. and NAKATA, A. (1983) Cloning and characterization of the *umu* operon responsible for inducible mutagenesis in *Escherichia coli*. *Gene* **23**, 167-174.
- SKIDMORE, C. J., DAVIS, M. I., GOODWIN, P. M., HALLDORSSON, H., LEWIS, P. J., SHALL, S. and ZIA'EE, A. A. (1979) The involvement of poly(ADP-ribose) polymerase in the degradation of NAD caused by  $\gamma$ -irradiation and N-methyl-N-nitrosourea. *Eur. J. Biochem.* **101**, 135-142.
- TIMSOM, J. (1977) Caffeine. *Mutation. Res.* **47**, 1-52.
- VARSHAVSKY, A. J., NEDOSPASCOV, S. A., BAKAYEV, V. V., BAKAYEVA, T. G. and GERGIEV, G. P. (1979) Histone-like proteins in the purified *Escherichia coli* deoxyribonucleoprotein. *Nucleic Acid Res.* **4**, 2725-2744.
- WITKIN, E. M. (1976) Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli*. *Bacteriol. Rev.* **40**, 869-907.