Jpn. J. Genet. (1987) 62, *pp.* 431-437

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

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(Received October 9, 1987)

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*$^+$ gene expression by UV was depressed by 3 ABA in both *uvrA* and wild-type strains. Prophage induction from λ-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*$^+$ 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^+, 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^+ 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 λ, 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 ($argF_{amber}$), Hs 30 R ($argF_{amber}$, *uvrA*) and NG 30 ($argF_{amber}$, *recA*). Two strains of λ-lysogenized *E. coli* W3110 (λ^+) and W3110 (λind^s) 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^+ 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^+ operator, and the $umuD^+$ and *umuC'-'lacZ* fusion genes (Shinagawa *et al.* 1983). Therefore, the UV induced activity of the umu^+ gene expression was determined by measuring the enzymatic activity of β-galactosidase, since $lacZ^+$ gene expression is controlled by the umu^+ 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. λ-dilution buffer and λ-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 3×10^8 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^2/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

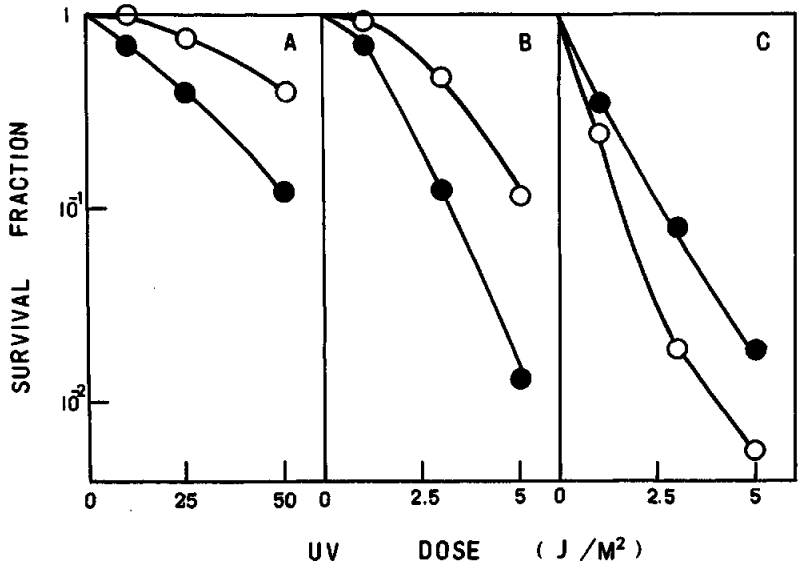

Fig. 1. Effect of 3 ABA on UV-lethality.
A, H/r 30 R (wild-type for repair); B, Hs 30 R (*uvrA*); C, NG 30 (*recA*). ○, without 3 ABA; ●, with 3 ABA.

counted after incubation for 20 hours at 37 °C.

Induced mutation. After UV-irradiation, the cells were washed and resuspended 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⁺ gene expression. UV irradiation was applied to cells of the KY series at a dosage of 10 J/m². The 3×10^8 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^+) and W 3110 (λind^s) at dosages of 30 J/m² and 3 J/m², 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.

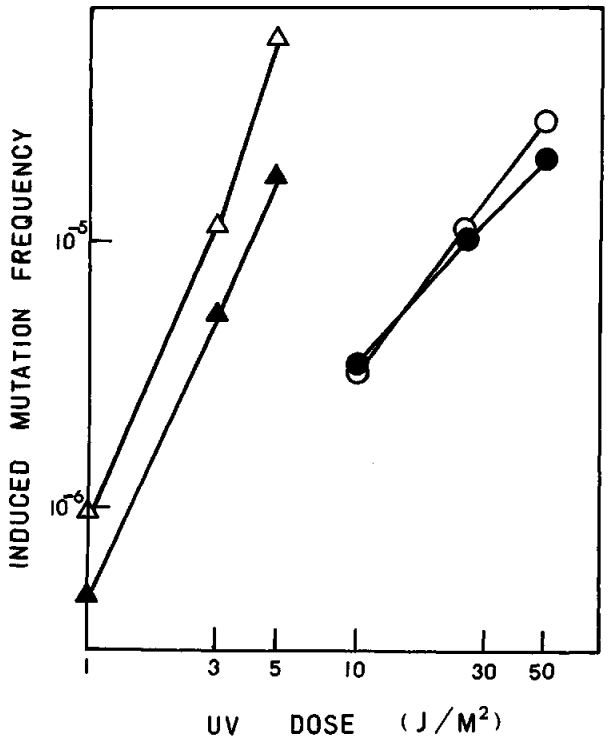

Fig. 2. Effect of 3 ABA on UV-induced mutation.
Circles, H/r 30 R (wild-type for repair); triangles, Hs 30 R (*uvrA*). Open symbols, without 3ABA; closed symbols, with 3 ABA.

3. RESULTS AND DISCUSSION

Effects of 3 ABA 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 3 ABA following UV irradiation enhanced cell killing in wild-type and *uvrA* strains. The sensitivity of each strain to UV as determined by its LD_{50}, was increased by 200% with 3 ABA. In contrast, the *recA* strain showed partial resistance to UV irradiation when 3 ABA was present. These results indicate that 3 ABA may inhibit certain DNA repair systems apart from excision repair.

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

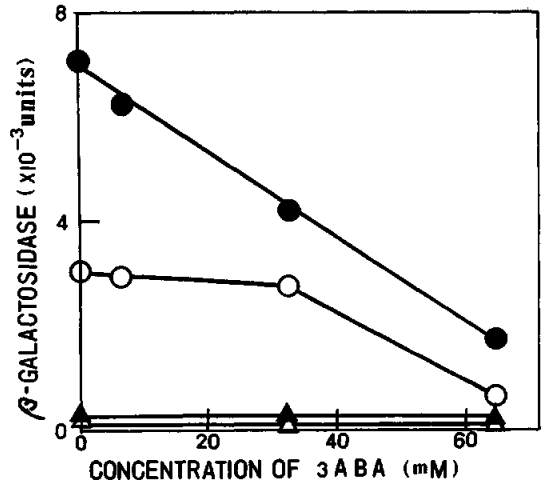

Fig. 3. Effect of 3 ABA on induction of the *umu*$^+$ gene expression.
Following UV irradiation, the cells were incubated for 2 h with 3 ABA at the indicated concentrations to induce the *umu*$^+$ gene expression. ○, KY 700 (wild-type for repair); ●, KY 706 (*uvrA*); ▲, KY 703 (*lexA*); △, KY 705 (*recA*).

gene expression (Fig. 3). Irradiation with UV could induce a high enzymatic activity of β-galactosidase, i.e., a high activity of *umu*$^+$ 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*$^+$ 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 λ-phages from λ-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 W 3110 (λ *ind*^{s}) and W3110 (λ$^+$), phage induction was markedly depressed by 3ABA 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,

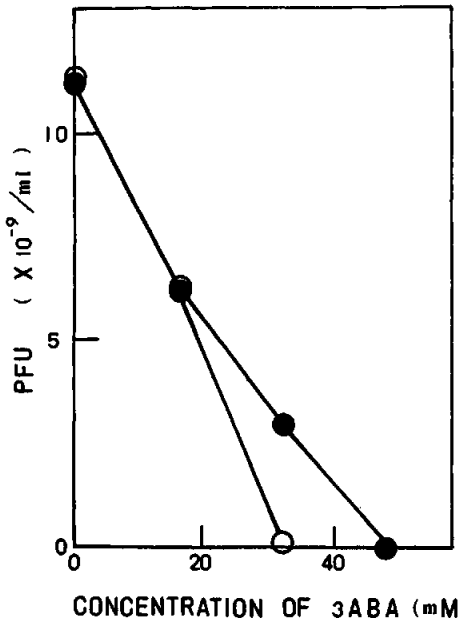

Fig. 4. Effect of 3 ABA on prophage induction.
Following UV irradiation, the cells were incubated for 100 minutes with 3 ABA at the indicated concentrations in order to induce prophage. ○, W 3110 (*λind*^{s}); ●, W 3110 (*λ*$^+$).

Timsom 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.

This work was supported by a Grant-in Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

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