

大腸菌における紫外線誘発 SOS 反応の3-アミノベンツアミドによる抑制

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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*⁺ 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 3ABA, 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. 3ABA, 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/m²/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 3ABA. This concentration of 3ABA is known to have almost no effect on the colony forming ability of *E. coli* cells. The number of colonies was

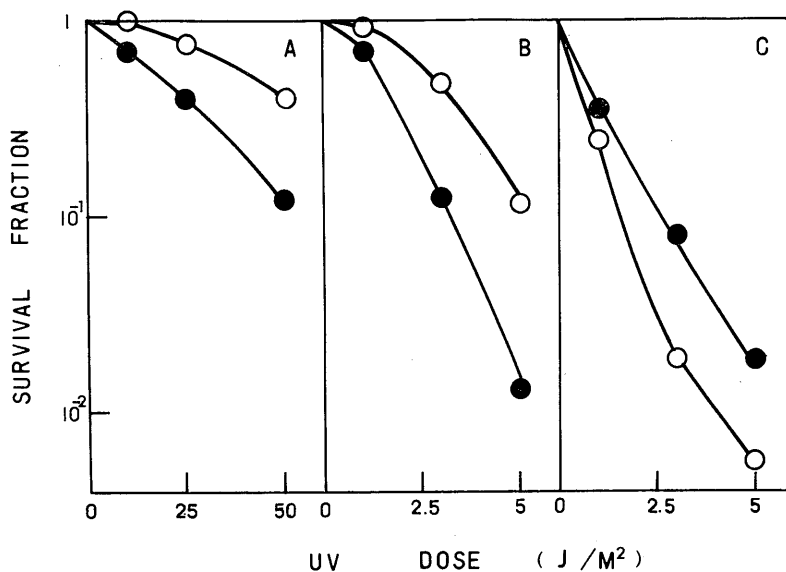


Fig. 1. Effect of 3 ABA on UV-lethality.

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*⁺ gene expression.* UV irradiation was applied to cells of the KY series at a dosage of 10 J/m². The 3 × 10⁸ 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 (*λ ind*^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.

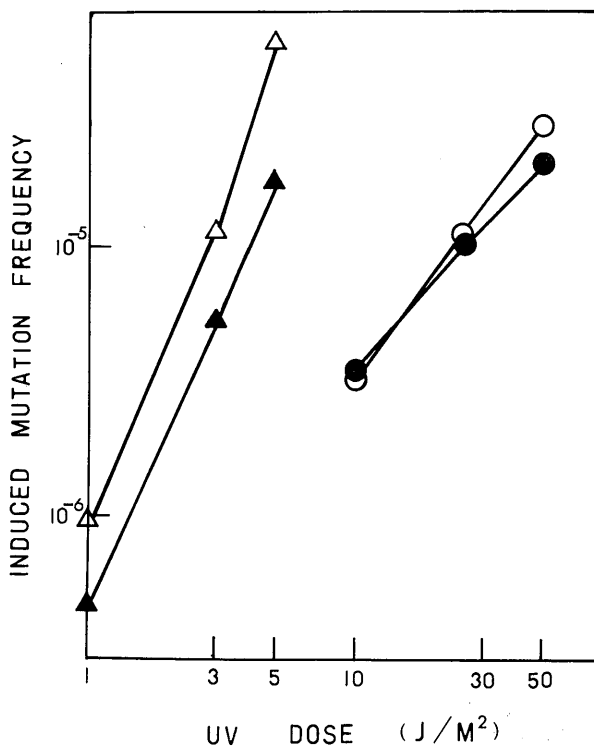


Fig. 2. Effect of 3 ABA on UV-induced mutation.

Circles, H/r 30 R (wild-type for repair); triangles, Hs 30 R (*uvrA*). Open symbols, without 3 ABA; 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 LD₅₀, 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*⁺

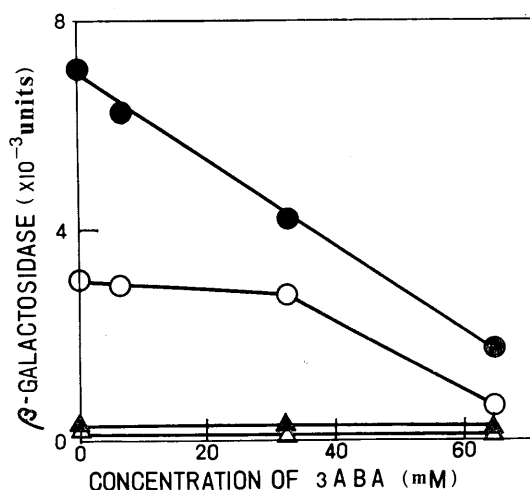


Fig. 3. Effect of 3 ABA on induction of the *umu*⁺ gene expression.

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 W 3110 (λ ⁺), 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,

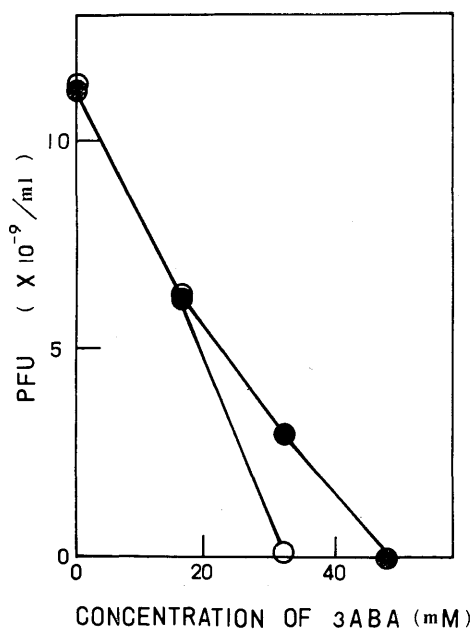


Fig. 4. Effect of 3 ABA on proophage induction.

Following UV irradiation, the cells were incubated for 100 minutes with 3 ABA at the indicated concentrations in order to induce proophage. ○, W3110 (λ^{ind^+}); ●, W3110 (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.

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