Effect of Alkylating Agents on the Expression of Inducible Genes of *Escherichia coli*

By J.-ALBERT VERICAT,* RICARDO GUERRERO AND JORDI BARBÉ

Department of Microbiology and Institute for Fundamental Biology, Autonomous University of Barcelona, Bellaterra (Barcelona), Spain

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Increasing doses of alkylating agents such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, diethyl sulphate and ethylmethane sulphonate cause an inhibition of the expression of the *recA* and *sfiA* genes of wild-type *Escherichia coli*. This behaviour was not observed in a *lexA56* mutant which has a defective LexA repressor that is unable to bind to the SOS operator. Furthermore, an *ada-1* mutant showed the same behaviour as the wild-type strain indicating that the adaptive proteins are not responsible for the inhibition of *recA* and *sfiA* at high doses of alkylating agents. These results suggest that the inhibitory effect of these alkylating agents may be found in the interaction between the LexA repressor and the control regions of *sfiA* and *recA*. On the other hand, high doses of either UV light or mitomycin C produced only a slight decrease in the induction of *recA* and *sfiA*, whereas bleomycin had no effect. The fact that a repressor structurally related to LexA repressor, such as LacI protein, showed the same behaviour as the LexA repressor when a Lac⁺ strain was treated with alkylating agents, suggests that these compounds can modify the binding abilities of repressors to DNA, producing a limited or even abolished release of repressors, and so decreasing the expression of inducible genes.

INTRODUCTION

When normal DNA replication is inhibited by different physical (UV or γ radiation), chemical (alkylnitrosoguanidines, mitomycin C, bleomycin), or physiological (thymine starvation, *dnaB* mutants growing at 42 °C) treatments, a set of cellular responses are induced that are known collectively as the SOS functions (Witkin, 1974; Walker, 1984). These functions include inhibition of cell division, cessation of respiration, induction and reactivation of prophages, error-prone repair activity and increased synthesis of RecA protein. This pleiotropic response depends on the *recA*, *lexA* and *ssb* genes (Little & Mount, 1982). It has been shown that LexA protein is the repressor of the SOS genes. The basal level of RecA protein is activated to a protease by an inducing signal after DNA damage. This signal is increased by the activity of the RecBC enzyme exonuclease V (Oishi *et al.*, 1979; Barbé *et al.*, 1985). Once activated, RecA protease can cleave LexA repressor and other SOS-related repressors, resulting in the expression of the SOS genes (Roberts *et al.*, 1978; Little *et al.*, 1980; Kenyon & Walker, 1981; Schendel *et al.*, 1982).

Previous studies have shown that the induction of several SOS functions is not an all-or-none process but a discriminated one (Guerrero & Barbé, 1982). This discriminated expression of the SOS system is specially clear in the case of alkylating agents such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), diethyl sulphate (DES) and ethylmethane sulphonate (EMS) (Barbé *et al.*, 1983). Thus, UV irradiation or bleomycin treatment induce cessation of respiration, filamentous cell growth and prophage induction. In contrast, the three alkylating

Abbreviations: MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; DES, diethyl sulphate; EMS, ethylmethane sulphonate; IPTG, isopropyl β -D-thiogalactopyranoside.

Table 1. *E. coli* K12 strains used in this work

Strain	Genotype	Source*
AB1157	<i>argE3 his4 thr1 proA2 leu6 thi1 lacY1 supE44 rpsL22</i>	M. Blanco
GC2375	As AB1157 but lysogenic for [λ d(<i>recA</i> :: <i>lacZ</i>)c <i>lind</i>]	R. D'Ari
GY4786	As AB1157 but lysogenic for [λ d(<i>sfiA</i> :: <i>lacZ</i>)c <i>lind</i>]	M. Blanco
PJ1	As AB1157 but <i>ada-1</i>	B. Sedgwick
UA4186	As PJ1 but lysogenic for [λ d(<i>recA</i> :: <i>lacZ</i>)c <i>lind</i>]	This laboratory
UA4201	As PJ1 but lysogenic for [λ d(<i>sfiA</i> :: <i>lacZ</i>)c <i>lind</i>]	This laboratory
IC372	As AB1157 but <i>lexA</i> (<i>Def</i>)56 <i>sfiA11 ilv</i>	M. Blanco
UA4209	As IC372 but lysogenic for [λ d(<i>recA</i> :: <i>lacZ</i>)c <i>lind</i>]	This work
UA4174	As IC372 but lysogenic for [λ d(<i>sfiA</i> :: <i>lacZ</i>)c <i>lind</i>]	This work
CGSC 5073	Wild-type	L. Archer
CGSC 808	HfrH <i>thi1 relA1 lacI22</i>	L. Archer

* Addresses: M. Blanco, Instituto de Investigaciones, Citológicas, Valencia, Spain; R. D'Ari, Institute Recherche Biologie Moléculaire, CNRS, Paris, France; B. Sedgwick, Imperial Cancer Research Foundation, Mill Hill, London, UK; L. Archer, Gulbenkian Institute, Oeiras, Portugal.

agents only induce cessation of respiration. All of these previous results suggest that this discriminated behaviour in the induction of SOS functions depends on the type of DNA lesion caused (Barbé *et al.*, 1983). We have also shown that this discriminated response is not related to the degree of non-specific DNA degradation or DNA synthesis arrest (Barbé *et al.*, 1983), the level of protein synthesis (Vericat *et al.*, 1984) or the amplification of RecA protein synthesis (Barbé *et al.*, 1985). The molecular basis of this discriminated induction of the SOS genes by the alkylating agents is not yet completely understood.

For this reason, and to determine the possible role of DNA alkylations on the discriminated induction of the SOS system, we have studied the induction of *recA* and *sfiA* when cells are treated with increasing doses of MNNG, DES or EMS, using UV light, bleomycin and mitomycin C as positive controls. We have also analysed the effect of all of these treatments on the induction of the *lac* operon by isopropyl β -D-galactopyranoside (IPTG).

METHODS

Bacterial strains and growth conditions. The bacterial strains used in this work are listed in Table 1. All are *Escherichia coli* K12 derivatives.

Cultures were grown at 37 °C with shaking in AB liquid minimal medium (Clark & Maaløe, 1967) supplemented with thiamin (10 μ g ml⁻¹), glucose (0.2%, w/v) or glycerol (0.2%, v/v), and Casamino acids (0.4%, w/v). In the case of mitomycin C or MNNG treatments the medium was adjusted to pH 5.5, which is optimal for the activity of both compounds (Cooke, 1981; Miller, 1972).

UV irradiation and lysogenization of bacterial strains. The procedures used were described by Barbé *et al.* (1985).

Chemical treatment. Cultures were grown to 2×10^8 cells ml⁻¹ in supplemented AB medium with shaking at 37 °C. Mutagen was then added and incubation was continued at 37 °C for 100 min. Samples were taken every 20 min.

Determination of β -galactosidase activity. *lacZ* expression in *E. coli* cells harbouring fusions between SOS genes and *lacZ*, or the expression of the *lac* operon induced by IPTG, were determined as described by Barbé *et al.* (1985). All the results are means of three independent experiments.

Chemicals. Casamino acids were from Oxoid. Vitamins and mineral salts were from Merck. Mitomycin C, diethyl sulphate, ethylmethane sulphonate, and IPTG were from Sigma. Bleomycin was from Almirall (Barcelona, Spain). MNNG, *o*-nitrophenyl β -D-galactopyranoside and phenyl β -D-galactopyranoside (to control the β -galactosidase constitutive phenotype; Smith & Sadler, 1971) were from Fluka. Ampicillin and streptomycin were a generous gift of Antibioticos, SA (León, Spain).

RESULTS

Expression of *recA* and *sfiA*

MNNG, DES and EMS completely inhibited *recA* expression in *E. coli* when present at concentrations exceeding 10 μ g ml⁻¹, 1 μ l ml⁻¹ and 10 μ l ml⁻¹, respectively (Fig. 1). UV light and mitomycin C caused only slight inhibition (Fig. 2*a, b*) and bleomycin had no effect (Fig. 2*c*).

Fig. 1. Induction of *recA* expression, measured as β -galactosidase activity, in cultures of the wild-type *E. coli* strain GC2375 after continuous treatment with MNNG (a), DES (b) or EMS (c). Concentrations of each were as follows: MNNG, 2 $\mu\text{g ml}^{-1}$ (■), 10 $\mu\text{g ml}^{-1}$ (○), 15 $\mu\text{g ml}^{-1}$ (▽) and 20 $\mu\text{g ml}^{-1}$ (▲); DES, 0.3 $\mu\text{l ml}^{-1}$ (■), 1 $\mu\text{l ml}^{-1}$ (○), 2 $\mu\text{l ml}^{-1}$ (▽) and 3 $\mu\text{l ml}^{-1}$ (▲); EMS, 3 $\mu\text{l ml}^{-1}$ (■), 10 $\mu\text{l ml}^{-1}$ (○), 20 $\mu\text{l ml}^{-1}$ (▽) and 30 $\mu\text{l ml}^{-1}$ (▲). The data are normalized with respect to the β -galactosidase activity present in this strain before treatment.

Fig. 2. Induction of *recA* expression, measured as β -galactosidase activity, in cultures of the wild-type *E. coli* strain GC2375 after irradiation with UV light (a), and after continuous treatment with mitomycin C (b) or bleomycin (c). Doses of each treatment were as follows: UV light, 5 J m⁻² (■), 10 J m⁻² (○), 20 J m⁻² (▽) and 40 J m⁻² (▲); mitomycin C, 5 $\mu\text{g ml}^{-1}$ (■), 10 $\mu\text{g ml}^{-1}$ (○), 20 $\mu\text{g ml}^{-1}$ (▽) and 40 $\mu\text{g ml}^{-1}$ (▲); bleomycin, 2.5 $\mu\text{g ml}^{-1}$ (■), 5 $\mu\text{g ml}^{-1}$ (○), 10 $\mu\text{g ml}^{-1}$ (▽) and 20 $\mu\text{g ml}^{-1}$ (▲). The data were treated as in Fig. 1.

Fig. 3. Induction of *sfiA* expression, measured as β -galactosidase activity, in cultures of the wild-type *E. coli* strain GY4786 after continuous treatment with MNNG (a), DES (b) or EMS (c). Doses, symbols and treatment of data are as in Fig. 1.

Fig. 4. Induction of *sfiA* expression, measured as β -galactosidase activity, in cultures of the wild-type *E. coli* strain GY4786 after irradiation with UV light (a), and after continuous treatment with mitomycin C (b) and bleomycin (c). Doses, symbols and treatment of data are as in Fig. 2.

Similar results were obtained in the study of the expression of the *sfiA*::*lacZ* fusion (Figs 3 and 4). Nevertheless, at doses of alkylating agents where no induction of *recA* was found (Fig. 1), there was a slight expression of *sfiA* (Fig. 3), confirming previous work (Moreau *et al.*, 1980; Barbé *et al.*, 1985) which showed that SOS genes could be induced without amplification of the RecA protein.

It has also been shown that the inhibitory effect of MNNG on the expression of the SOS functions is not related to a non-specific effect on the synthesis of proteins (Vericat *et al.*, 1984), which suggests that alkylating agents can interfere specifically with the synthesis and activity of SOS-related proteins. For this reason, we tested the induction of *recA* and *sfiA* in a *lexA56*

Fig. 5. Effect of treatment with MNNG (a), DES (b) and EMS (c), either 10 min before (●) or 10 min after (△) activation of the *lac* operon, measured as relative units of β -galactosidase activity, in *E. coli* strain CGSC 5073 induced by IPTG addition. The β -galactosidase activity of a culture induced by IPTG but without mutagenic treatment was used as a control (○). Concentrations of alkylating agents were as follows: MNNG, 20 $\mu\text{g ml}^{-1}$; DES, 3 $\mu\text{g ml}^{-1}$; and EMS, 30 $\mu\text{l ml}^{-1}$. The data were treated as in Fig. 1.

mutant, which is constitutive for the SOS functions because its LexA repressor lacks DNA-operator-binding activity. Results obtained showed that alkylating agents did not affect the level of transcription of either *recA* or *sfiA* in the *lexA56* strain (data not shown).

It is known that the adaptive response induced by alkylating agents interferes with the induction of the SOS response (Jeggo *et al.*, 1977). To ascertain whether high concentrations of alkylating agents such as MNNG, DES or EMS could interfere with the expression of the SOS genes through the adaptive response, we did similar experiments with an *ada-1* mutant, harbouring either the *recA::lacZ* or *sfiA::lacZ* fusions. Results obtained showed that the dose-related pattern of expression of the SOS genes in *E. coli* cultures treated with alkylating agents is independent of the intracellular level of adaptive proteins (data not shown).

Induction of the *lac* operon by IPTG

The above results suggested that there was some kind of *ada*-independent interaction between the action of high doses of alkylating agents and the expression of SOS genes. Furthermore, it has been shown that the LacI protein, the repressor of the lactose operon, has the same three-dimensional conformation as other repressors, such as the LexA repressor, Lambda cI repressor and other binding proteins such as Cro protein (Pabo & Lewis, 1982; Pabo *et al.*, 1982). For these reasons, and to determine if the decreased expression of the SOS response after addition of alkylating agents was a specific event related to the SOS genes or a non-specific feature related to inducible genes in general, we studied the induction of the *lac* operon by IPTG under conditions where there was no expression of *recA* and *sfiA*. Fig. 5 shows the effect of MNNG, DES and EMS on β -galactosidase synthesis in the wild-type *E. coli* strain CGSC 5073. When cells were treated with IPTG before addition of the alkylating agents there was a significant increase in β -galactosidase synthesis. In contrast, when IPTG was added to the medium after the alkylating agents there was no synthesis of β -galactosidase. However, when the same experiments were done with the constitutive *lac* strain (CGSC 808), the alkylating agents had no effect on β -galactosidase synthesis (data not shown). On the other hand, cells treated with mitomycin C or UV light only showed a negligible inhibition of β -galactosidase synthesis independent of the time of activation of the *lac* operon; bleomycin did not affect β -galactosidase synthesis (Fig. 6).

Fig. 6. Effect of treatment with UV light (a), mitomycin C (b) and bleomycin (c), either 10 min before (●) or 10 min after (△) activation of the *lac* operon, measured as relative units of β -galactosidase activity, in *E. coli* strain CGSC 5073 induced by IPTG addition. The β -galactosidase activity of a culture induced by IPTG but without mutagenic treatment was used as a control (○). Doses of each treatment were as follows: UV light, 40 J m^{-2} ; mitomycin C, $40 \mu\text{g ml}^{-1}$; and bleomycin, $20 \mu\text{g ml}^{-1}$. The data were treated as in Fig. 1.

DISCUSSION

Results presented in this work show that high doses of alkylating agents decrease or avoid the induction of *recA*, *sfiA* and *lacZ* (Figs 1, 3 and 5), whereas low doses stimulate slightly the expression of the two SOS genes. These results are in agreement with previous data showing that adaptive doses of MNNG induce a *recA::trp* fusion (Smith *et al.*, 1983) and that *sfiA* is induced by MNNG and EMS (Ohta *et al.*, 1984). On the other hand, higher doses of UV light or mitomycin C, which cause structural DNA alteration and few DNA alkylations, produce only a slight decrease in the level of expression of *recA*, *sfiA* and *lacZ* (Figs 2, 4 and 6). Bleomycin, which causes only single stranded DNA gaps (D'Andrea & Haseltine, 1978), does not produce any decrease in the induction of *recA*, *sfiA* and *lacZ* (Figs 2, 4 and 6). These results suggest the existence of some kind of relationship between the extent of alkylation of DNA and the level of induction of some inducible genes. However, the decrease in the expression of *recA* and *sfiA* by the alkylating agents cannot be attributed to any non-specific effect on their transcription, because the treatments do not affect the induction of *recA* and *sfiA* in the *lexA56* mutant, which has a LexA repressor that lacks DNA-binding ability. Furthermore, in the case of the *lacZ* gene, when the system is completely induced in a constitutive Lac strain (CGSC 808), the alkylating agents did not prevent its expression.

This fact suggests that the inhibitory effect of the alkylating agents has to be related to some kind of interaction between LexA or LacI repressors and their operator-binding regions. Since UV light can fix proteins covalently to DNA, so the DNA polymerase of *E. coli* may be bound to DNA *in vitro* to an extent that is directly proportional to UV dose (Markovitz, 1972). Several authors have suggested that adducts between thymine and basic amino acids are very important in this binding (Simič & Dizdaroğlu, 1985). Thus, it is possible to propose that high doses of UV light could link, or increase the affinity of, LacI, LexA and other SOS-related repressors to their operator binding targets, resulting respectively in a complete, or partial, decrease in the expression of inducible genes. It seems that this phenomenon is very general and that other proteins are bound to DNA by UV light (Kunkel & Martinson, 1978). Nevertheless, the situation remains more obscure for the alkylating treatments. It has been shown that many

DNA-binding proteins have very similar domains. The Cro protein and the cI and c2 phage-repressors (lambda and P22) have more than 30% homology in α -helix regions associated with protein-DNA recognition (Anderson *et al.*, 1982). Homology has also been demonstrated between the lambda repressor and many other proteins such as the repressor of phage 434, LacI and GalI (repressors of *lac* and *gal* operons), LexA protein, Cro protein from phage 434, cII protein from lambda and the CAP protein (Matthews *et al.*, 1983; Steitz *et al.*, 1983); the homologies are about 25% (Sauer *et al.*, 1982a). It has also been shown that some repressors such as cI from lambda, c2 from P22, cI from 434 and LexA are cleaved by RecA protease between the same amino acids (Sauer *et al.*, 1982b).

Thus, the fact that MNNG, DES and EMS modify preferentially guanines in DNA (Auerbach, 1976; Helène, 1977; Hoffmann, 1980; Lawley, 1974; Lawley & Orr, 1970) and that alkyl nitrosoguanidines originate alkylations with the same efficiency in DNA and proteins (Yoda *et al.*, 1982), giving rise to reactive centres that could produce covalent bonds between DNA and proteins, suggest that alkylating agents, when modifying DNA, could produce adducts between DNA and repressors. It is interesting to note that in the regulatory region of lambda there are eight guanines, three of which participate in the binding of the protein to DNA (Sauer *et al.*, 1979). It has also been shown that substituting different bases for thymine in synthetic oligonucleotides results in either important decreases or increases in the affinity of LacI or CAP proteins for DNA (Lin & Riggs, 1972, 1976). This would avoid the release of the repressor and, as a consequence, the transcription of inducible genes.

Our results support this hypothesis. Increasing doses of alkylating agents inhibited expression of the inducible genes tested (*recA*, *sfiA* and *lacZ*); treatments that produce either some structural modifications or alkylations (UV light or mitomycin C) caused a slight decrease in the expression of these genes. However, non-alkylating compounds such as bleomycin did not reduce the activity of *recA*, *sfiA* or *lacZ*. Finally, and in agreement with the above hypothesis, neither alkylating agents nor UV light nor mitomycin C affected the expression of the genes studied in their respective constitutive mutants.

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