Effect of Adenine, Cytidine and Guanosine on the Expression of the SOS System in *Escherichia coli*

By MONTSERRAT LLAGOSTERA,* RICARDO GUERRERO,
ANTONIO VILLAVERDE AND JORGE BARBÉ

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

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Addition of cytidine or guanosine to UV-irradiated cells of a RecA⁺ strain of *Escherichia coli* did not produce any effect on the induction of two SOS functions: inhibition of cell division and expression of the *umuC* gene. Under the same conditions adenine gave a slight increase in the induction of these two responses. In a RecA441 mutant growing at 42 °C, both cytidine and guanosine inhibited these SOS functions, whereas adenine produced a large increase in their expression. Moreover, the ATP concentration of the RecA441 mutant at 42 °C showed a decrease which occurred earlier in the cells growing in the presence of cytidine or guanosine than in the absence of either compound. Adenine induced an increase of about three times the initial ATP concentration of this mutant at 42 °C which dropped quickly after 10 min. Neither cytidine nor guanosine increased the evolution of cellular ATP in UV-irradiated cells of the RecA⁺ strain, whereas adenine had only a slight positive effect. However, in UV-irradiated RecA⁺ cells with and without adenine, ATP levels dropped quickly to the initial value after 20 min. These data suggest that the influence of adenine, cytidine and guanosine on the expression of the RecA441 phenotype at 42 °C may be due to alteration of the cellular ATP concentration of this mutant.

INTRODUCTION

Damage to DNA in *Escherichia coli* by radiation, chemical carcinogens or any treatment that inhibits DNA replication induces a set of cellular activities known as the SOS system (for a review, see Little & Mount, 1982). These SOS functions include inhibition of cell division, induction and reactivation of prophages, cessation of respiration and massive synthesis of RecA protein. Nevertheless, some SOS functions may be induced by some treatments but not by others (Guerrero & Barbé, 1982; Barbé *et al.*, 1983a), showing that expression of the SOS system is not an all-or-none response. However, all of these SOS functions depend on the *recA*, *lexA* and *ssb* genes. Recent biochemical studies on the RecA protein have shown that it can act as a specific protease which, after activation by some signal such as single strand DNA fragments generated by DNA damage, cleaves the repressors that control phage induction (Roberts *et al.*, 1978). The RecA protein also cleaves the product of the *lexA* gene, which regulates the rate of transcription of the different genes involved in the SOS system (Little *et al.*, 1981). Among the *recA* mutants which have been isolated and characterized, the *tif-1* mutant, renamed *recA441*, is one of the most extensively studied. This mutant can express RecA functions at 42 °C even in the absence of DNA damage or arrested DNA synthesis, whereas at 32 °C it behaves like the wild-type RecA (Gudas & Mount, 1977).

The expression of SOS functions in the *recA441* mutant at 42 °C may be affected by different conditions. The presence of adenine in the growth medium increases the SOS response of this strain at 42 °C (Kirby *et al.*, 1967) whereas cytidine or guanosine inhibit induction of the RecA441 phenotype (George *et al.*, 1975). Nevertheless, no explanation for the effect of these

Table 1 *Strains of Escherichia coli K12 used in this work*

Strain	Genotype	Reference
AB1157	<i>argE3 his4 thr1 proA2 leu6 thi1 supE44 rpsL</i>	Howard-Flanders & Theriot (1962)
JM12	As AB1157, but <i>recA441 supE</i>	Castellazzi <i>et al.</i> (1972)
GW1000	As AB1157, but <i>Pro⁺Δlac(U169) galK2 ilv(ts) recA441 sfiA11/pSE140 (Km^r Φ(<i>umuC lacZ</i>))</i>	Elledge & Walker (1983)

compounds on UV-mediated induction of SOS functions in RecA⁺ cells has hitherto been reported. We have studied the influence of adenine, cytidine and guanosine on the ATP concentration of the UV-irradiated RecA⁺ strain and in the RecA441 mutant growing at 42 °C, and suggest a molecular explanation for the effect of adenine, cytidine and guanosine on the expression of the RecA441 phenotype.

METHODS

Bacteria and growth conditions. Bacterial strains used in this work are listed in Table 1. Cultures were grown at 32 °C or 37 °C with shaking. The minimal salts medium AB (Clark & Maaløe, 1967) was supplemented with thiamin (10 µg ml⁻¹), glucose (0.2%, w/v) and Casamino acids (0.4%, w/v).

UV irradiation. Cultures were grown to 2×10^8 cells ml⁻¹ in supplemented AB medium, and were irradiated in a glass Petri dish (10 cm diameter) with a General Electric GY1578 germicidal lamp, at a rate of 0.5 J m⁻² s⁻¹ (determined with a Latarjet dosimeter). After irradiation cells were centrifuged, resuspended in the same volume of supplemented AB medium, and incubated at the desired temperature.

Determination of cell size. Cell size was determined with a Coulter counter ZBI equipped with a 30 µm orifice, as previously described (Barbé *et al.*, 1983a).

Determination of the intracellular ATP concentration. Cell samples of 1 ml were added to 4 ml 0.025 M-Tris/HCl buffer (pH 7.75) and boiled in a water bath for 5 min. The suspension was centrifuged for 10 min at 4 °C and 8000 g. ATP present in the supernatant was immediately measured by the firefly luciferin-luciferase assay (Chapman *et al.*, 1971).

β-Galactosidase assay. This was done as described by Miller (1972). Enzyme concentrations (U ml⁻¹) were calculated from the formula given by Casaregola *et al.* (1982): $[(A_{420} - 1.75 A_{550})/0.0075 t] \times (\text{reaction vol./sample vol.})$ where t is the time of reaction (min), the A_{550} corrects for the scattering by residual bacterial debris and 0.0075 is the A_{420} of 1 nm-*o*-nitrophenol. The reaction volume was 1.75 ml and the sample volume was 0.1 ml.

Chemicals. Casamino acids were obtained from Oxoid. Vitamins and mineral salts were purchased from Merck, and nucleosides, nucleotides and firefly luciferin-luciferase assay components from Sigma.

RESULTS

Inhibition of cell division

Changes of cell volume of RecA⁺ strain after UV irradiation in the presence or absence of either adenine, cytidine or guanosine are shown in Fig. 1. Under these conditions, an increase in cell volume occurred in all cultures. Nevertheless, filamentation found in the UV-irradiated cultures treated with adenine is slightly higher than that shown by the cells with cytidine or guanosine added. Furthermore, UV-irradiated cells treated with either cytidine or guanosine showed the same pattern of filamentation as the cultures without any addition.

In contrast to these results, adenine produced a significant increase in the pattern of filamentation of the *recA441* mutant growing at 42 °C, whereas both cytidine and guanosine prevented the temperature-dependent inhibition of cell division (Fig. 2). However, the effect of adenine on the UV-mediated filamentation of the RecA⁺ strain is considerably less than that induced by this nucleotide on the temperature-dependent filamentation of the *recA441* mutant.

Induction of the umuC gene expression

Mutations at the *umuC* locus make cells of *E. coli* non-mutable by UV-irradiation and chemical mutagens (Kato & Shinoura, 1977). Thus, the *umuC* gene product is the best candidate

Fig. 1. Distribution of cell volumes of UV-irradiated RecA⁺ strain growing in the absence (a) or in the presence of adenine (100 $\mu\text{g ml}^{-1}$) (b), cytidine (300 $\mu\text{g ml}^{-1}$) (c), or guanosine (350 $\mu\text{g ml}^{-1}$) (d). Measurements were made at intervals after irradiation.

Fig. 2. Distribution of cell volumes of RecA441 strain growing at 42 °C in the absence (a) or in the presence of adenine (100 $\mu\text{g ml}^{-1}$) (b), cytidine (300 $\mu\text{g ml}^{-1}$) (c), or guanosine (350 $\mu\text{g ml}^{-1}$) (d).

for a protein that plays a key mechanistic role in the process of error-prone repair. For this reason we studied the induction of the expression of the *umuC* gene in the presence of adenine, cytidine or guanosine following UV-irradiation as well as temperature treatment in a RecA441 SfiA⁻ mutant. For this we used a low-copy-number plasmid, pSE140, which has a fusion between the *umuC* and *lac* operons (Elledge & Walker, 1983). Adenine caused a slight increase in

Fig. 3

Fig. 4

Fig. 3. Induction of *umuC* gene expression, indicated by increased β -galactosidase, in cultures of strain GW1000 growing at 32 °C after UV-irradiation and in the presence of adenine (100 $\mu\text{g ml}^{-1}$) (○), cytosine (300 $\mu\text{g ml}^{-1}$) (●) or guanosine (350 $\mu\text{g ml}^{-1}$) (■). *umuC* expression of UV-irradiated (□) and non-irradiated (△) cultures of the same strain growing without any addition is shown as a control.

Fig. 4. Induction of *umuC* gene expression, indicated by increased β -galactosidase, in cultures of strain GW1000 at 42 °C and in the presence of adenine (100 $\mu\text{g ml}^{-1}$) (○), cytosine (300 $\mu\text{g ml}^{-1}$) (□) or guanosine (350 $\mu\text{g ml}^{-1}$) (■). *umuC* expression of cultures of the same strain growing either at 32 °C (△) or 42 °C (●) without any addition is shown as a control.

the UV-mediated induction of *umuC* gene in this strain growing at 32 °C, whereas cultures treated with either cytosine or guanosine showed the same degree of *umuC* induction as the irradiated controls (Fig. 3). Nevertheless, the effect of adenine, cytosine and guanosine on the induction of the *umuC* gene in the RecA441 mutant growing at 42 °C was similar to that produced by the same compounds on the temperature-induced inhibition of cell division in this mutant. Thus, adenine gave an increase in the expression of *umuC* gene whereas cytosine and guanosine inhibited expression (Fig. 4).

Changes in cell ATP concentration

In previous studies (Barbé *et al.*, 1983b) we have shown that after UV-irradiation the RecA⁺ cells show an increase in ATP concentration during the first 20 min and that afterwards ATP drops to the concentration of non-treated cells. This last ATP decrease is RecA⁺ and LexA⁺ dependent. In the same way, the RecA441 mutant shows a decrease in the ATP concentration 10 min after the temperature shift to 42 °C. These data led us to study the changes in the cellular ATP concentration following adenine, cytosine or guanosine addition in all UV-irradiated and non-irradiated RecA⁺ strains and in the RecA441 mutant growing at 42 °C. Fig. 5 shows that adenine produced during the first 20 min an increase of about twofold in the initial ATP concentration of non-irradiated RecA⁺ cells, which afterwards dropped to about the initial value. Cytidine and guanosine induced a decrease of the ATP pool of the RecA⁺ cells. Furthermore, in UV-irradiated RecA⁺ cells, adenine only induced a slight increase in the ATP pool (Fig. 6), whereas cytosine and guanosine had a negligible effect.

The change in ATP concentration in the RecA441 mutant growing at 42 °C in the presence or absence of adenine, cytosine or guanosine is shown in Fig. 7. Adenine produced a threefold increase in the concentration of ATP, which dropped below the original concentration 10 min after adenine addition. Cytidine and guanosine produced a decrease in the ATP concentration which was greater than that shown by the untreated culture growing at the restrictive temperature.

Fig. 5

Fig. 6

Fig. 7

Fig. 5. Changes in cellular ATP in a *RecA*⁺ strain growing in the presence of either adenine ($100 \mu\text{g ml}^{-1}$) (●), cytidine ($300 \mu\text{g ml}^{-1}$) (□) or guanosine ($350 \mu\text{g ml}^{-1}$) (■). The ATP concentration of an untreated culture (○) is shown as a control.

Fig. 6. Changes in cellular ATP in a UV-irradiated *RecA*⁺ strain growing in the presence of either adenine ($100 \mu\text{g ml}^{-1}$) (●), cytidine ($300 \mu\text{g ml}^{-1}$) (□) or guanosine ($350 \mu\text{g ml}^{-1}$) (■). The ATP concentrations of both irradiated (○) and non-irradiated (△) *RecA*⁺ cultures growing without any addition are shown as controls.

Fig. 7. Changes in cellular ATP in a *RecA441* mutant growing at 42°C in the presence of either adenine ($100 \mu\text{g ml}^{-1}$) (●), cytidine ($300 \mu\text{g ml}^{-1}$) (□) or guanosine ($350 \mu\text{g ml}^{-1}$) (■). The ATP concentrations of the *RecA441* mutant growing either at 42°C (○) or 32°C (△), both without any addition are shown as controls.

DISCUSSION

We present evidence to indicate that cytidine and guanosine do not have any effect on the UV-mediated expression of the two SOS functions studied here: induction of the *umuC* gene and inhibition of cell division. Furthermore, adenine produced a slight increase in the expression of these SOS functions after UV-irradiation. Nevertheless, in the *RecA441* mutant growing at 42°C , addition of adenine, cytidine or guanosine produced a large increase and also a strong inhibition of the same SOS functions, in agreement with previous data reported by different authors (Goldthwait & Jacob, 1964). Tessman & Peterson (1980) suggested that the *RecA441* form of the *RecA* protein could be an allosteric enzyme which binds both negative and positive modulators. Thus, adenine or its derivatives would be positive modulators and cytidine or guanosine would be negative ones. However, our results show that adenine produces an important increase in the ATP concentration of both non-irradiated *RecA*⁺ strain and *RecA441* mutant at 42°C and that cytidine and guanosine decrease the concentration of ATP. All these data suggest that the effect of these compounds on the expression of SOS functions in the *RecA441* mutant growing at 42°C could be due to variations in the intracellular ATP concentration, since this mutant hydrolyses ATP to induce the SOS system (Barbé *et al.*, 1983b). Thus, adenine could stimulate SOS system induction of the *RecA441* mutant because, in its presence, the cells have an ATP concentration higher than when adenine has not been added (Fig. 7). Related to this, non-irradiated *RecA*⁺ cells growing in the presence of adenine show a drop in the ATP concentration about 20 min after the compound is added (Fig. 5), whereas in the *RecA441* mutant growing at 42°C , ATP decreases 10 min after adenine addition (Fig. 7). This is in agreement with the time that the mutant takes to hydrolyse the ATP when the SOS functions are induced at the restrictive temperature. Likewise, cytidine and guanosine could

inhibit the SOS functions in the RecA441 mutant since both compounds deplete the cellular ATP pool. Under these conditions, the activated RecA441 protein would be unable to cleave the LexA repressor because this reaction *in vivo* requires ATP hydrolysis (Barbé *et al.*, 1983*b*). The poor adenine-mediated stimulation of SOS expression of RecA⁺ UV-irradiated cells must be attributed to the slight increase of the ATP concentration induced by adenine (Fig. 6). Furthermore, cytidine and guanosine did not have any effect on the UV-mediated induction of SOS functions because neither compound affected the ATP concentration of irradiated cells (Fig. 6). In this context, the possible effect of cytidine and guanosine on the ATP pool of UV-irradiated RecA⁺ cells must be overcome by the strong UV-dependent ATP increase which is produced in the irradiated cells by metabolism of DNA degradation products (Barbé *et al.*, 1983*b*; Guerrero *et al.*, 1984). However, our hypothesis also explains the observation (Kirby *et al.*, 1967) that UV-irradiation reverses the cytidine- and guanosine-mediated inhibition of constitutive expression of SOS functions at 42 °C in the RecA441 mutant.

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