Isolation and Characterization of a Mutant of *Escherichia coli* K12 Synthesizing DNA Polymerase I and Endonuclease I Constitutively

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A mutant of *Escherichia coli* K12, highly resistant to ultraviolet radiation, has been isolated. Preliminary tests show that this mutant is also resistant to mitomycin C, nalidixic acid, fluorouracil and thymineless death. The mutant strain apparently repairs its damaged DNA more efficiently than wild-type *E. coli* K12 strains and, to do so, constitutively produces 35 times more DNA polymerase I and 12 times more endonuclease I than the wild-type strain.

INTRODUCTION

Wild-type strains of *Escherichia coli* are relatively resistant to ultraviolet radiation (u.v.) since they have several DNA repair mechanisms (for review, see Grossman *et al.*, 1975). Some R factors and colicin factors may also confer partial protection to their host cell, *E. coli*, against the bactericidal effect of u.v. (Siccardi, 1969). Although a mutant strain, *E. coli* P6 derived from *E. coli* B/r, was found to have enhanced resistance to X-rays (Zelle & Ogg, 1957), there is no report of any *E. coli* K12 mutant with increased resistance to u.v. The X-ray resistant cells were larger than the parent strain cells, weighed about three times more, contained approximately three times more DNA and RNA, and were resistant to phage T1 (Zelle & Ogg, 1957). Genetic analysis of this P6 strain showed that a single mutation was responsible for the pleiotropic phenotype (Kvetkas *et al.*, 1970).

In this paper we report the isolation and preliminary characterization of a mutant of *E. coli* K12 with enhanced resistance to u.v. killing, thymineless death, mitomycin C, nalidixic acid and fluorouracil compared with its parent strain. It would appear that the mutant strain has gained these abilities as a result of synthesizing DNA polymerase I and endonuclease I constitutively.

METHODS

Bacterial strains. *Escherichia coli* Hfr KL14, AB1157 (Bachmann, 1972), SA236 (u.v. super-resistant derivative of KL14), SA229 (*thy* derivative of AB1157) and SA242 (u.v. super-resistant derivative of SA229) were used.

Isolation of u.v. super-resistant (UVS-r) mutant. The mutant strain SA236 was isolated from Hfr KL14 by exposing bacteria (approx. 5×10^7 per plate) on nutrient agar to a u.v. dose of 180 J m^{-2} followed by

analysis of survivors for u.v. resistance. Strain SA236 tolerated a heavier dose of u.v. than its parent strain, KL14.

The UVS-r character was transferred from strain SA236 to SA229 by conjugation selecting for His⁺ recombinants.

Characterization of UVS-r mutant. Strains KL14 (wild-type) and SA236 (UVS-r) were tested qualitatively for resistance to mitomycin C (MC), nalidixic acid (NAL), fluorouracil (FU), hydrogen peroxide (H₂O₂) and chloramphenicol (CAP). This was done by adding 0.5 ml of freshly grown bacterial cultures in nutrient broth (each having the same A₄₅₀) to 4.5 ml of 0.6% nutrient agar and then overlaying the bacteria on nutrient agar plates. Analytical discs (12.7 mm diam.; Carl Schleicher & Schüll Co., Dassel, Germany) were then impregnated with solutions of the inhibitors (MC, FU and CAP, 1 mg ml⁻¹; NAL, 5 mg ml⁻¹; H₂O₂, 10 mg ml⁻¹) and placed on the bacterial lawn. After overnight incubation, the inhibition zones were measured.

Measurement of thymineless death. The rate of thymineless death was measured as described earlier (Ahmad, 1980).

Enzyme assays. For enzyme analysis, freshly grown bacteria in double-strength nutrient broth were washed with 50 mM-Tris/HCl, pH 7.5, containing 1 mM-MgCl₂ and broken by sonication (20 kHz, 5 A, 4 × 1 min in ice) at 4 g wet wt packed cells per 20 ml buffer A [50 mM-Tris/HCl, pH 7.5, containing 1 mM-MgCl₂, 0.5 mM-Na₂EDTA, 10 mM-2-mercaptoethanol, 0.1 mM-phenylmethylsulphonyl fluoride and 5% (w/v) glycerol]. Following centrifugation at 40000 *g* for 1 h at 4 °C the clarified cell extracts were dialysed against 10 mM-potassium phosphate, pH 7.5, and analysed for enzyme activities as below, and for protein using Lowry's method.

DNA polymerase I was assayed using 'activated' calf thymus DNA as described by Englund (1971); tRNA (40 µg ml⁻¹) was added to inhibit endonuclease I activity. Exonuclease I and exonuclease III were assayed as described by Lehman (1966) and by the phosphatase assay of Richardson (1966), respectively, with tRNA (40 µg ml⁻¹) added to inhibit endonuclease I activity in both assays. DNA ligase was assayed by adenylation essentially as described by Gellert (1971) after dialysis against buffer A to remove endogenous NAD. Endonuclease I was assayed essentially as described by De Waard & Lehman (1966), the activity being taken as the difference between the value for the assay including RNAase and that excluding RNAase but including tRNA (40 µg ml⁻¹). Exonuclease II activity was assayed as described by Obinata & Mijuno (1968) but using *p*-nitrophenylthymidine-5-phosphate as substrate. In all cases activity is expressed as units (mg protein)⁻¹ and units are as described in the assay references. tRNA was included in the assays for DNA polymerase I, exonuclease I and exonuclease III to eliminate the effect that different levels of endonuclease I in the two strains (see Table 1) might have on the assay for these enzymes.

RESULTS AND DISCUSSION

After exposure to a u.v. dose of 180 J m⁻² (time of exposure, 2 min), 0.0067% of the wild-type and 1.85% of UVS-r cells survived (Fig. 1). The measured Dose Enhancement Factor (DEF) value (Hartman & Eisenstark, 1978) of the resistant mutant was 1.64. Further analysis of the mutant strain showed that it had lost the ability to grow on minimal medium on which the parent strain could grow. Upon testing for its new nutritional requirements, no amino acids and/or vitamins could be found to satisfy its growth needs; instead, growth occurred only on complex media such as nutrient agar or broth.

Enzyme analysis of the cell extracts of both KL14 (wild-type) and SA236 (UVS-r) showed significant differences in the activities of enzymes associated with nucleic acid

Table 1. *DNA repair enzyme activities in strains KL14 (wild-type) and SA236 (UVS-r)*

Enzyme units are as used by previous authors (see Methods).

Enzyme	Enzyme activity [units (mg protein) ⁻¹]	
	KL14 (wild-type)	SA236 (UVS-r)
DNA polymerase I	3.2	116.0
Exonuclease I	10.7	12.2
Exonuclease II	3.8	3.3
Exonuclease III	36.0	81.0
Endonuclease I	14.0	170.0
DNA ligase	2.2	2.0

Fig. 1

Fig. 2

Fig. 1. Survival of KL14 (wild-type) (▼) and SA236 (UVS-r) (▽) at various u.v. doses. Cultures in M9 salts medium (Clowes & Hayes, 1968) were treated by superimposing exposures of 30 s (45 J m^{-2}) each. After each 30 s exposure samples were removed, diluted and plated on nutrient agar.

Fig. 2. Thymineless death in SA242 (UVS-r) (△) and SA229 (wild-type) (▲) strains.

Table 2. *Inhibition of growth of strains KL14 (wild-type) and SA236 (UVS-r) by various agents*

Inhibitor*	Concn (mg ml^{-1})	Zone of inhibition (mm)	
		KL14 (wild-type)	SA236 (UVS-r)
MC	1	22	18
NAL	5	37	32
FU	1	19	None
CAP	1	27	27
H ₂ O ₂	10	19	19

* MC, Mitomycin C; NAL, nalidixic acid; FU, fluorouracil; CAP, chloramphenicol.

synthesis, metabolism and repair (Table 1). In the UVS-r strain, SA236, DNA polymerase I activity was increased 35-fold, endonuclease I activity was increased 12-fold and exonuclease III activity was increased 2-fold compared with the wild-type strain, KL14. It is likely that the large elevation in both DNA polymerase I and endonuclease I activities in SA236 are two of the factors responsible for the super u.v. resistance of this strain, because constitutive levels of these enzymes should result in rapid and efficient repair of u.v.-damaged DNA. The activities of exonuclease I, exonuclease II and DNA ligase, on the other hand, remained unchanged.

The UVS-r strain, SA236, remained as sensitive as the wild-type, KL14, to CAP and H₂O₂, but was more resistant to FU, MC and NAL (Table 2). Since FU, MC, NAL, H₂O₂ and thymine starvation are known DNA damaging agents (Ananthaswamy & Eisenstark, 1977; Cozzarelli, 1977; Nakayama & Hanawalt, 1975) and since the mutant strain has simultaneously enhanced its resistance to most of these agents, it can be assumed that the

mutant bacteria repair damaged DNA more efficiently than do the wild-type organisms. The lack of resistance to H_2O_2 may be because activity of DNA ligase in the mutant strain is no higher than in the wild-type parent strain, hence the single-strand DNA breaks induced by H_2O_2 (Ananthaswamy & Eisenstark, 1977) are repaired no more efficiently.

To test the mutant strain for thymineless death, the putative UVS-r mutation(s) was first transferred by conjugation to a *thy his* strain, SA229, selecting for His⁺ recombinants. One of the *thy his*⁺ derivatives, SA242, which showed enhanced resistance to u.v. killing (results not presented), was examined for tolerance to thymineless death. It may be seen (Fig. 2) that, in addition to UVS-r properties, strain SA242 was also more resistant to thymineless death than its recipient parent strain, SA229.

Measurement of the growth of SA229 (wild-type) and SA242 (UVS-r) in minimal synthetic medium (results not presented) and microscopic examination showed that the mutant strain did not differ from the wild-type parent strain either in growth rate or morphology. This is a distinct difference from the P6 X-ray resistant-strain described previously (Zelle & Ogg, 1957). Further genetic analysis of the UVS-r strain is in progress.

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