Loss of Ultraviolet Light Protection and Enhanced Ultraviolet Light-induced Mutability in *Pseudomonas aeruginosa* Carrying Mutant R Plasmids

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Two mutants of the R plasmid pMG2, selected on the basis of loss of enhanced ultraviolet light (u.v.)-induced mutability, no longer protected a wild type, a *uvr rec*⁺ type mutant, or a *polA* type mutant of *Pseudomonas aeruginosa* against the lethal effects of u.v. For one of the R plasmid mutants tested, pPL2, a plasmid-determined DNA polymerase was no longer detected. The properties of these mutants support the hypothesis that a plasmid gene function responsible for enhanced u.v.-induced mutability also confers u.v.-protection on the host cells. Furthermore a plasmid-determined DNA polymerase plays a major role in producing these effects.

On the basis of the effects of sodium arsenite on plasmid u.v.-protection and enhanced u.v.-induced mutability in strain TRPF1, it is proposed that a *recA*-dependent, arsenite-sensitive repair pathway is present in *P. aeruginosa*.

INTRODUCTION

Several R plasmids, including R-Utrecht (MacPhee, 1973) and R46 (Mortelmans & Stocker, 1976) in *Salmonella typhimurium* as well as pMG2 in *Pseudomonas aeruginosa* (Lehrbach *et al.*, 1977), have the ability to protect host cells against the lethal effects of ultraviolet irradiation while increasing the susceptibility of the host to the mutagenic effects of ultraviolet light (u.v.). These studies suggest that the plasmids carry a gene, or genes, which act to increase the extent of repair of u.v.-damaged host DNA. Furthermore, since an intact *recA*⁺ gene is essential for these plasmids to exert their effect on the u.v. response of cells (MacPhee, 1973; Lehrbach *et al.*, 1977), it is possible that the plasmid gene product(s) is involved in an error-prone repair pathway.

The pleiotropic effects of a *recA* mutation in *S. typhimurium* (MacPhee, 1970), *Escherichia coli* (Clark & Margulies, 1965) and *P. aeruginosa* (Chandler & Krishnapillai, 1974) implicate this gene in the control of a number of different pathways. Repair inhibitors, such as chloramphenicol (Sedgwick, 1975), sodium arsenite (Rossman, Meyr & Troll, 1975) and other chemicals (Smith, 1971), that inhibit a *rec*-dependent function have been used to separate and define various *rec*-dependent pathways. For example, the chloramphenicol-sensitive component of post-replication repair in *E. coli* apparently identifies an inducible error-prone mechanism (Sedgwick, 1975). Sodium arsenite has been postulated to inhibit a pathway controlled by the *recA* gene, which involves the *exr*⁺ gene and performs the majority of the *recA*-dependent repair in *E. coli* B (Rossman *et al.*, 1975).

The u.v.-protection function of the R plasmid pMG2 is dependent on a *recA* gene (Lehrbach *et al.*, 1977). Also, studies on cells deficient in a DNA polymerase I-type function in *P. aeruginosa* suggest that an R plasmid gene product of pMG2 may participate in the filling of single-strand gaps introduced during the repair of u.v.-damaged DNA. To investigate the extent to which an arsenite-sensitive repair pathway may be responsible for

Table 1. *Bacterial strains*

Strain no.	Strain characteristics*	Reference
PAO1	prototrophic, <i>chl-2</i>	Holloway (1955)
TRPFI	<i>trpF1</i>	Calhoun, Pierson & Jensen (1973)
GMB112	<i>polA3</i>	Lehrbach <i>et al.</i> (1976)
GMA918	<i>uvr-81</i>	Kung & Lee (1973)
PU21(pMG2)	<i>ilvB112 leu-1 str-1 rif</i> FP ⁻ pMG2 (Gm Sm Su Hg Phi E79 UV)	Jacoby (1974)
TRPFI(pPL2)	<i>trpF1</i> pPL2 (Gm Sm Su Hg Phi E79 UV ⁻)	This paper
TRPFI(pPL3)	<i>trpF1</i> pPL3 (Gm Sm Su Hg Phi E79 UV ⁻)	This paper

* Antibiotic resistances: *chl*, chloramphenicol; *rif*, rifampin; Gm, gentamicin; Sm or *str*, streptomycin; Su, sulphonamide. Hg, Resistance to mercuric ions. Phi, Interference with phage propagation. UV, U.v. protection or resistance.

the observed u.v.-protection and enhanced u.v.-induced mutability of pMG2, we have tested the effects of sodium arsenite on the u.v.-survival and u.v.-induced mutability of strains carrying pMG2. Mutants of pMG2 were selected on the basis of enhanced u.v.-induced mutability. The properties of these mutants are described and a further definition of pathways involved in plasmid-mediated repair in *P. aeruginosa* is proposed.

METHODS

Bacteria. The standard wild-type strain is *Pseudomonas aeruginosa* PAO1 (ATCC15692) (Holloway, 1955). Strains used in this study are listed in Table 1. R plasmid pMG2 is a P-2 group plasmid originally characterized by Jacoby (1974). R plasmid mutant derivatives of pMG2 have been named according to Novick *et al.* (1976).

General culture procedures, N-methyl-N'-nitro-N-nitrosoguanidine (NTG) mutagenesis, u.v.-irradiation and u.v.-survival curves. These techniques have been previously described (Kung & Lee, 1973).

U.v.-mutagenesis. To determine the frequency of u.v.-induced mutations, the yield of Trp⁺ back mutations of strain TRPFI following various u.v. doses was scored. Samples (0.1 ml) of irradiated and control cell suspensions were spread on minimal agar medium supplemented with 2.5 % (v/v) nutrient broth (Oxoid, CM67). For each u.v. dose and for the spontaneous level, three plates were used. Survival following each u.v. dose was determined by plating on the same medium. Sodium arsenite was used at a final concentration of 1 mM.

Assay for DNA polymerase activity. The method used was that of Lehrbach, Kung & Lee (1976).

R plasmid transfer. Exponentially growing cultures of donor and recipient cells were mixed in a ratio of 1:10 respectively in nutrient broth containing potassium nitrate at 4 mg ml⁻¹. The mixture was incubated for 2 h without shaking in a glass Petri plate at 37 °C. R⁺ recipients were selected by appropriate genotype and drug resistance (sulphonamide resistance was not tested).

Isolation of R plasmid mutants. NTG-mutagenized clones of TRPFI (pMG2) were cultured in nutrient broth. Equal volumes of saline suspensions of these derivatives were then spotted on two broth-supplemented minimal medium agar plates. One plate was irradiated with 10 J m⁻² and both plates were incubated for 48 h at 37 °C. Two of 750 clones tested were found to lack the enhanced u.v.-induced mutability associated with pMG2.

RESULTS

The two mutant R plasmids isolated in this study were selected by an on-the-plate test for loss of enhanced u.v.-induced mutability. They were designated pPL2 and pPL3 and retained resistance to gentamicin, streptomycin and mercuric ions as well as the ability to be transferred to various strains of *P. aeruginosa*. These mutant plasmids were tested for their ability to alter u.v.-survival in various genetic backgrounds and to enhance u.v.-induced mutability in strain TRPFI. One of them, pPL2, was assayed for a plasmid-determined DNA polymerase I-type activity.

Fig. 1. U.v.-survival curves of strain TRPF1 (○) containing R plasmid pMG2 (●) and mutant R plasmids pPL2 (▽) and pPL3 (△).

Effect of the R plasmid pMG2 and its derivatives pPL2 and pPL3 on u.v.-survival and u.v.-induced mutation in TRPF1

The plasmid pMG2 confers u.v.-protection to strain TRPF1. U.v.-survival curves of TRPF1 containing pPL2 or pPL3 did not show the extended shoulder associated with the u.v.-resistance of TRPF1(pMG2) (Fig. 1). At higher u.v. doses a slight protective effect was observed for the mutant R plasmids in the TRPF1 background which may indicate a slight residual plasmid repair function in these mutants.

Plasmid pMG2 substantially increased the mutation frequency after u.v.-irradiation whereas the mutant R plasmids pPL2 and pPL3 showed significantly lower u.v.-induced mutation frequencies in the TRPF1 strain (Table 2). The spontaneous back mutation was similarly affected.

*Effect of R plasmids pMG2, pPL2 and pPL3 on u.v.-survival in host-cell reactivation (*hcr*)- and polymerase (*polA*)-deficient mutants*

The presence of pMG2 protects the wild type and a variety of repair-deficient strains, including *hcr* and *polA* mutants of *P. aeruginosa*, from u.v.-damage (Lehrbach *et al.*, 1977). To determine whether bacteria containing pPL2 and pPL3 had altered u.v.-sensitivity, these plasmids were transferred to a host-cell reactivation (*hcr*)- deficient strain GMA918 (Kung & Lee, 1973) and to a polymerase (*polA*)-deficient strain GMB112 (Lehrbach *et al.*, 1976). U.v.-survival curves for these strains (Fig. 2*a, b*) showed that pPL2 and pPL3 have lost the ability to protect the *hcr* and *polA* strains against the lethal effects of u.v. A similar loss of u.v.-protection was shown for the wild-type strain (PAO1) from which the repair-deficient strains were derived (unpublished data).

DNA polymerase activity associated with pMG2

A plasmid-determined DNA polymerase I-type activity (i.e. assayable in crude sonicated extracts) has previously been studied in *P. aeruginosa* strain GMB112 carrying pMG2 (Lehrbach *et al.*, 1977). To test if this polymerase function was concomitantly lost in the mutant

Fig. 2. U.v.-survival curves of strains (a) GMA918 (*uvr-81*) and (b) GMB112 (*polA3*), with and without R plasmids: ○, no plasmid; ●, pMG2; ▽, pPL2; △, pPL3.

Table 2. U.v.-induction of *Trp*⁺ revertants in strains TRPFI, TRPFI(pMG2), TRPFI(pPL2) and TRPFI(pPL3)

Strain	Medium*	Spontaneous frequency per 10 ⁷ cells	Induced frequency per 10 ⁷ survivors† at u.v. dose (J m ⁻²):		
			5	10	20
TRPFI	MB	3	8.6 (64)	8.4 (31)	36 (8)
TRPFI(pMG2)	MB	12	240 (90)	392 (73)	526 (61)
TRPFI(pPL2)	MB	4	23 (85)	33 (43)	48 (10)
TRPFI(pPL3)	MB	4	27 (79)	56 (43)	94 (9)
TRPFI	MB + sodium arsenite	0.8	1 (80)	3.6 (21)	3 (9)
TRPFI(pMG2)	MB + sodium arsenite	1.6	14 (60)	56 (31)	59 (10)

* MB, Minimal medium supplemented with 2.5 % (v/v) nutrient broth. Sodium arsenite was used at a final concentration of 1 mM.

† Numbers show the mean frequency of *Trp*⁺ revertants per 10⁷ survivors, corrected for spontaneous mutations by subtracting the mean frequency of revertants on the non-irradiated plates (see Methods); numbers in parentheses show the percentage survival.

R plasmids (pPL2 and pPL3) which were selected on the basis of loss of enhanced u.v.-mutability, DNA polymerase activity was determined in a *polA* mutant (GMB112) harbouring one of the mutant plasmids pPL2 (Fig. 3). Crude extracts of GMB112 showed no detectable polymerizing activity whereas GMB112(pMG2) showed a significant increase in polymerase activity. This increase was not observed for GMB112(pPL2).

Fig. 3

Fig. 3. Assay of polymerase activity of strains: ■, PAOI; ○, GMB112; ▲, GMB112(pMG2); ▽, GMB112(pPL2).

Fig. 4

Fig. 4. U.v.-survival curves of strains TRPFI (○, ●) and TRPFI(pMG2) (□, ■) in the presence (●, ■) or absence (○, □) of 1 mM-sodium arsenite.

Effect of sodium arsenite on u.v.-protection and enhanced u.v.-induced mutability of pMG2

Studies using *E. coli* B have shown that sodium arsenite inhibits a *recA*-dependent step in the repair of u.v.-irradiated DNA (Rossman *et al.*, 1975). The arsenite-sensitive step contributes to the survival of cells following u.v.-irradiation because u.v.-irradiated *E. coli* (WP2) is more sensitive to u.v. when post-treated with sodium arsenite, whereas arsenite has no effect on the u.v.-survival of *recA* mutants (Rossman *et al.*, 1975).

Since the u.v.-protective effect of pMG2 is dependent on the *recA* gene in *P. aeruginosa* the effect of a sublethal concentration of sodium arsenite (1 mM) in the plating medium following u.v.-irradiation was examined in strain TRPFI (wild type for repair) (Table 2, Fig. 4). The presence of sodium arsenite eliminated the extended shoulder of the u.v.-survival curve of TRPFI(pMG2) reducing the u.v.-survival of TRPFI(pMG2) to that of u.v.-irradiated strain TRPFI in the presence of sodium arsenite. U.v.-induced mutability of TRPFI(pMG2) was also significantly reduced.

DISCUSSION

The properties of the mutant *R* plasmids pPL2 and pPL3 which were selected on the basis of the loss of enhanced u.v.-mutability can be summarized as follows: (i) a significant reduction of u.v.-induced back mutation frequencies in TRPFI(pPL2) and TRPFI(pPL3) compared with TRPFI(pMG2); (ii) loss of enhanced spontaneous mutability in TRPFI(pPL2) and TRPFI(pPL3) compared with TRPFI(pMG2); (iii) loss of u.v.-protection in wild-type (TRPFI), *polA* (GMB112) and *hcr* (GMA918) host cells compared with the same strains carrying the *R* plasmid pMG2; (iv) loss of a plasmid-determined DNA polymerase I-type activity (tested only for mutant *R* plasmid pPL2).

These properties support the hypothesis that a plasmid gene function responsible for enhanced u.v.-induced mutability also confers u.v.-protection on the host cell containing pMG2.

The slight protective effect of pPL2 and pPL3 observed at higher u.v. doses in TRPFI (Fig. 1) and the increased levels of u.v.-induced back mutation (Trp⁺) of TRPFI(pPL2) and TRPFI(pPL3) (Table 2) could indicate the presence of further plasmid-mediated repair functions. It has been postulated that u.v.-protection produced by the R plasmid R46 in *E. coli* involves an inducible gene product which is possibly a nuclease and is dependent on a functional host *recA* gene for expression (Tweats *et al.*, 1976). The presence of such an enzyme associated with pMG2 has not been examined, but such repair nucleases could play a part in plasmid-mediated u.v.-resistance in *P. aeruginosa*. However, on the basis of the properties of R plasmid mutant pPL2, the presence of a plasmid-determined DNA polymerase activity clearly plays a major role in altering the u.v.-survival and u.v.-induced mutability of bacteria carrying pMG2. It is possible that this plasmid-determined gene product may participate in the *recA*-dependent error-prone repair of u.v.-damaged host DNA in *P. aeruginosa*.

Both u.v.-survival and u.v.-induced mutability of TRPFI(pMG2) are greatly reduced in the presence of sublethal concentrations of sodium arsenite (1 mM). These results suggest that the increased u.v.-repair capacity of bacteria carrying pMG2 is due to a *recA*-dependent, arsenite-sensitive step in DNA repair.

The association of repair genes as part of an R plasmid genome may have been in response to several agents which act to create structural modifications to DNA which resemble stages in the repair of u.v.- or γ -irradiated DNA (i.e. DNA strand breaks). The ability of antibiotics such as nalidixic acid (Gudas & Pardee, 1976), bleomycin (Kuo & Haidle, 1974) and phleomycin (Nakayama, 1975) to inhibit DNA synthesis and stimulate DNA degradation to produce DNA strand gaps is analogous to DNA strand breaks produced after excision of u.v.-induced pyrimidine dimers or breaks produced by γ -irradiation or treatment with methyl methanesulphonate or NTG. Strains of *P. aeruginosa* carrying the plasmid pMG2 are protected against the lethal effects of u.v.- and γ -irradiation, methyl methanesulphonate and NTG.

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