Absence of Ultraviolet-inducible DNA Polymerase I-like Activity in Escherichia coli Strains Harbouring R Plasmids

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No DNA polymerase I-like activity was found associated with the ultraviolet (u.v.)-protecting plasmids R205, R46 or pKM101 in either uninduced or u.v.-induced wild-type or DNA polymerase I-deficient strains of Escherichia coli. Nor was any plasmid-associated polymerase activity detectable in similar systems containing u.v.-irradiated DNA as template. However, plasmids R205, R46 and pKM101 still increased survival and mutagenesis of the polymerase I-deficient E. coli strain after u.v. irradiation.

INTRODUCTION

Certain R plasmids reduce the susceptibility of host bacteria to ultraviolet (u.v.) irradiation whilst increasing its mutagenic effect (Mortelmans & Stocker, 1976). The frequency of spontaneous mutation may also be increased in R+ strains (Mortelmans & Stocker, 1979; Fowler et al., 1979). These plasmid-mediated functions occur in the absence of host-cell uvrA, lig and polA gene activities, which are primarily involved in short patch excision repair of DNA; they are also independent of the recB, recC and recF genes associated with recombination repair. However, no plasmid-associated u.v. protection or mutagenesis is found in recA strains (Tweets et al., 1976; Walker, 1977). A requirement for the lexA+ genotype appears to be variable, depending on the lexA allele tested (Monti-Bragadin et al., 1976). The recA and lexA genes control a complex inducible DNA repair pathway (the 'SOS' system) which after u.v. irradiation leads to delayed cell division, mutagenesis, induction of prophage, mutagenic Weigle-reactivation of phage and increased synthesis of protein X (Witkin, 1976; Volkert et al., 1979). RecA and lexA cells are also highly sensitive to u.v. and X-irradiation (Moody et al., 1973).

The DNA repair system encoded by R plasmid pKM101 appears to have both constitutive and inducible elements. Walker (1977) reported that unirradiated pKM101-containing cells were more efficient than R− cells in the repair of u.v.-irradiated λ phage. He suggested that this might be due to a constitutively expressed plasmid-coded protein that increased the activity of the cells' error-prone repair system. UmuC mutants of Escherichia coli are specifically deficient in u.v.-induced mutagenesis, other recA+ and lexA+-dependent functions being unaffected (Kato & Shinoura, 1977) and Walker & Dobson (1979) found that u.v. irradiation of a umuC strain containing pKM101 increased the Weigle-reactivation of u.v.-irradiated λ. It was therefore proposed that at least a proportion of the pKM101-mediated activity must be inducible by u.v. However, the mechanisms by which such plasmids raise the DNA repair capacity of host cells and increase both spontaneous and induced mutation frequencies have yet to be elucidated.

Mortelmans & Stocker (1979) have inferred that plasmid R46 (the parent plasmid from which pKM101 was derived) possesses at least two genes involved in DNA repair and mutagenesis. One,  uwp, increases the capacity of the cell for error-prone DNA repair and the
other codes for the ‘mutator’ activity which increases spontaneous mutation frequency in the host. Various functions have been proposed for the up-coded protein. Tweats et al. (1976) suggested that a nuclease believed to be responsible for plasmid elimination during thymine starvation (Tweats et al., 1974) might also be the plasmid protein responsible for u.v. protection. Lackey et al. (1977) found a constitutive endonuclease associated with pKM101 in Salmonella typhimurium, but this activity was still present in cells containing plasmid derivatives deficient in both repair and mutagenesis functions. A DNA polymerase I-like activity has been found associated with the u.v.-protecting plasmid R205 when present in a DNA polymerase I-deficient (polA) strain of S. typhimurium (MacPhee, 1974). Lehrbach et al. (1977a) also detected a DNA polymerase I-like activity associated with plasmid pMG2 in a polA strain of Pseudomonas aeruginosa; this activity was absent from the same strain containing mutants of pMG2 unable to protect from u.v. or to enhance u.v.-induced mutagenesis (Lehrbach et al., 1977b). These latter results prompted the examination of other u.v.-protecting plasmids for an associated DNA polymerase I-like activity. However, Kronish & Walker (1979) failed to detect any DNA polymerase I-like activity associated with the plasmids R205 and pKM101 when present either in a polA+ strain of E. coli or in a series of polA derivatives of this strain. Upton & Pinney (1979) were also unable to detect any DNA polymerase I-like activity mediated by the plasmids R205, R46 and pKM101 in polA+ or polA strains of E. coli. Since it appears that at least a proportion of plasmid-mediated repair activity is inducible by u.v. and that even the constitutive plasmid-coded products might interact with the inducible SOS system in an E. coli host (Walker & Dobson, 1979), we have now examined u.v.-induced cells for plasmid-associated DNA polymerase I-like activity.

METHODS

Organisms. Escherichia coli strain MM300 (ile thy rha lac polA+) (Monk & Kinross, 1972), its derivative JG138 (thi rha lac polA) (Monk et al., 1971) and strain AB1157 (thr leu pro his thi arg lac gal ara xyl mtl tsx rpsL sup-37) (Bachmann, 1972) were used.

Plasmids. The three N incompatibility group plasmids R205 (MacPhee, 1972), R46 (Anderson & Datta, 1965) and its derivative pKM101 (Mortelmans & Stocker, 1979) were transferred into the above strains from other laboratory strains of E. coli.

Media. Nutrient broth no. 2 (code CM67) and blood agar base (code CM55), both obtained from Oxoid, were supplemented with thyme (Koch-Light) at 60 µg ml−1. Minimal medium (DM) was as described by Davis & Mingioli (1950) solidified with 1.5% (w/v) Lab M agar (London Analytical and Bacteriological Media).

Biochemicals. Deoxyribonucleotide triphosphates were obtained from P-L Biochemicals, bovine serum albumin from Armour Pharmaceutical Co., calf thymus DNA from Sigma, and [methyl-3H]thymidine triphosphate from The Radiochemical Centre, Amersham.

Ultraviolet irradiation. Solutions and cell suspensions were irradiated in 5 cm diameter glass Petri dishes, with stirring, under a Hanovia model 12 low-pressure mercury lamp, which emitted light at 254 nm. The lamp height was adjusted so that the dose rate was either 1.1 or 2.8 J m−2 s−1. Dosimetry was determined using a model J-225 Blak-Ray UV meter (Ultra-violet Products, San Gabriel, Calif., U.S.A.).

Activated DNA template. A solution of calf thymus DNA (2 mg ml−1) and bovine serum albumin (0.5 mg ml−1) in buffer (50 mM-Tris/HCl, pH 7.4, containing 10 mM-MgSO4) was treated with pancreatic DNAase (0.1 µg ml−1) for 15 min at 37°C. The DNAase was then inactivated by heating at 80°C for 5 min. This preparation was diluted with buffer to give 500 µg DNA ml−1 and stored at −20°C.

Preparation of cell extracts. Cultures were grown with shaking at 37°C in nutrient broth to a concentration of 3–5 × 108 organisms ml−1. After chilling to 4°C the cells were harvested by centrifugation and resuspended to give 20-fold concentration in cold buffer. A crude cell extract was prepared by ultrasonic disintegration using an MSE 150 W ultrasonic disintegrator (mark 2) for a total of 90 s at a peak-to-peak amplitude of 6 µm. The extract was then centrifuged at 12,500 g for 20 min at 4°C to remove cell debris and the supernatant was stored at −20°C. For u.v.-induced preparations, cells were grown and harvested by the above method except that they were resuspended to give 10-fold concentration and then exposed to a u.v. dose of 14 J m−2. The irradiated cells were immediately diluted to the original volume with fresh warm nutrient broth and incubated for a further 40 min at 37°C with shaking, after which the normal procedure for the preparation of cell extracts was followed. Viable counts of cell suspensions were estimated by dilution in DM salts solution (DM base) and spreading 0.1 ml samples on nutrient agar plates. Colonies were counted after incubation overnight at 37°C.
Absence of R plasmid DNA polymerases

Assay for DNA polymerase I-like activity. DNA polymerase activity was assayed in reaction mixtures (250 µl) that contained either (1) 50 mM-Tris/HCl, pH 7.4, 10 mM-MgSO₄, 50 µg activated DNA, 20 µM-dATP, 20 µM-dCTP, 20 µM-dGTP, 20 µM-dTTP, 100 µl cell extract and 2.5 µCi [methy-³H]dTTP ml⁻¹ (50 Ci mmol⁻¹; 1.85 TBq mmol⁻¹), or (2) 50 mM-Tris/HCl, pH 7.4, 10 mM-MgSO₄, 5 µg unactivated DNA that had been subjected to ultrasonic disintegration for 90 s at a peak-to-peak amplitude of 6 µm. 20 µM-dATP, 20 µM-dCTP, 20 µM-dGTP, 20 µM-dTTP, 100 µl cell extract and 2.5 µCi [methy-³H]dTTP ml⁻¹ (50 Ci mmol⁻¹; 1.85 TBq mmol⁻¹). Samples (50 µl) were taken from the reaction mixture and spotted on 2 x 2 cm squares of Whatman 3MM chromatography paper. After absorption these were placed in ice-cold 5% (w/v) trichloroacetic acid (TCA)/1% (w/v) tetra-sodium pyrophosphate for 30 min. The samples were then washed twice for 15 min in cold TCA/pyrophosphate and once for 5 min in each of absolute alcohol, absolute alcohol/ether (1:1, v/v) and ether. Radioactivity was determined in a toluene-based scintillation fluid using a Packard Tri-Carb liquid scintillation counter.

Measurement of survival and mutagenesis after u.v. irradiation. U.v.-induced mutagenesis was measured in the E. coli MM300 (ilv thy rha lac polA+) and E. coli JG138 (thy rha lac polA) strains in the absence of any plasmid and with plasmids R46, R205 or pKM101 present. It was determined as the reversion frequency of the lac mutation to lac⁺. Cultures grown overnight in nutrient broth at 37 °C were washed and resuspended in DM base at 2 x 10⁸ organisms ml⁻¹. Samples (0-1 ml) of such suspensions were spread on DM agar containing 60 µg thymine ml⁻¹, 1% (v/v) nutrient broth and 0-3% (w/v) lactose; only cells that have reverted to lac⁺ produce colonies on this medium. These plates were either incubated immediately to determine spontaneous reversion of the lac mutation, or u.v.-irradiated with 5-5 J m⁻² before incubation to measure u.v.-induced reversion. The frequency of reversion to lac⁺ induced by U.V. irradiation was calculated by subtracting the number of spontaneous revertants per plate from the number of u.v.-induced revertants per plate and dividing by the number of viable bacteria per plate. The original cell suspensions were diluted in DM base and 0-1 ml samples were plated on nutrient agar to determine the initial concentration of viable cells. Similar plates were also exposed to u.v. irradiation to measure the increase in survival produced by the presence of the plasmids in the polA and polA⁺ E. coli strains. Incubation was for 48 h at 37 °C.

RESULTS

DNA polymerization in uninduced strains

DNA synthesis occurred in reaction mixtures containing cell extracts of the wild-type E. coli polA⁺ strains but not in mixtures containing extracts of the polA strain (Fig. 1). The polA

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**Fig. 1.** Incorporation of [³H]dTTP into acid-insoluble material by cell extracts of uninduced E. coli MM300 (polA⁺) strains [R⁻ (○), R205 (□), R46 (●), pKM101 (■)] and uninduced E. coli JG138 (polA) strains [R⁻, R205, R46 or pKM101 (△)]. Reaction mixture no. 2 was used.

**Fig. 2.** Incorporation of [³H]dTTP into acid-insoluble material by cell extracts of u.v.-induced E. coli MM300 (polA⁺) strains [R⁻ (○), R205 (□), R46 (●), pKM101 (■)] and u.v.-induced E. coli JG138 (polA) strains [R⁻, R205, R46 or pKM101 (△)]. Reaction mixture no. 1 was used.
Table 1. Post-u.v. survival and mutagenesis in E. coli strains MM300 (polA+) and JG138 (polA) harbouring plasmids R205, R46 and pKM101

<table>
<thead>
<tr>
<th>Strain</th>
<th>Post-u.v. survival</th>
<th>Mutagenesis (lac* revertants per 10^8 survivors after u.v. dose of 5-5 J m^-2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U.v. dose (J m^-2)</td>
<td>Survival (%)</td>
</tr>
<tr>
<td>polA⁺ R⁻</td>
<td>112</td>
<td>0.0089</td>
</tr>
<tr>
<td>R205</td>
<td>112</td>
<td>0.087</td>
</tr>
<tr>
<td>R46</td>
<td>112</td>
<td>0.053</td>
</tr>
<tr>
<td>pKM101</td>
<td>112</td>
<td>0.050</td>
</tr>
<tr>
<td>polA R⁻</td>
<td>33</td>
<td>0.0020</td>
</tr>
<tr>
<td>R205</td>
<td>33</td>
<td>0.0051</td>
</tr>
<tr>
<td>R46</td>
<td>33</td>
<td>0.0051</td>
</tr>
<tr>
<td>pKM101</td>
<td>33</td>
<td>0.0052</td>
</tr>
</tbody>
</table>

mutation does not affect the activities of host DNA polymerase II or DNA polymerase III (Gefeller et al., 1971), and the results in Fig. 1 therefore indicate that the assay system used was specific for DNA polymerase I-like synthesis.

No increase in DNA polymerization was observed in the polA⁺ or polA strains when plasmids R205, R46 or pKM101 were present (Fig. 1). These results confirm those of Kronish & Walker (1979) who also used polA⁺ and polA strains of E. coli. However, such results differ from those of MacPhee (1974) using plasmid R205 in Salmonella typhimurium polA and of Lehrbach et al. (1977a) with plasmid pMG2 in Pseudomonas aeruginosa polA. Both found that significant DNA polymerase I-like activity was conferred on the respective polA strains by the particular plasmid used.

Plasmid-mediated u.v. protection and u.v.-induced mutagenesis were produced in the polA E. coli strain (Table 1) showing that the uuv gene product was still expressed in this species, even though the putative error-prone DNA polymerase activity found associated with uuv plasmids in S. typhimurium and P. aeruginosa could not be demonstrated. Therefore, since Walker & Dobson (1979) have shown that at least a proportion of plasmid-mediated repair activity is inducible by u.v., DNA polymerization experiments were repeated using extracts of u.v.-induced cultures.

DNA polymerization in u.v.-induced strains

No DNA polymerase I-like activity associated with plasmids R205, R46 and pKM101 was detectable in cell extracts of u.v.-induced polA⁺ or polA E. coli strains. The presence of these plasmids in the induced polA⁺ strain neither increased nor decreased the level of DNA synthesis from that measured in the R⁻ strain (Fig. 2), and no DNA synthesis at all could be detected in induced R⁺ and R⁻ polA strains (Fig. 2). It therefore appears that plasmids R205, R46 and pKM101 code for neither a constitutive nor an inducible DNA polymerase I-like enzyme. It may also be concluded that no new DNA polymerase I-like enzyme is synthesized by the host in response to u.v. irradiation since there is no activity in extracts of the induced R⁻ polA cultures.

Polymerization on u.v.-irradiated DNA templates

If plasmid-coded mutagenic DNA repair were due to an error-prone DNA polymerase I-like enzyme, its mode of action might be to insert non-complementary bases opposite pyrimidine dimers. These are the major lesions produced in DNA by u.v. light; they are non-coding in DNA replication and therefore normally block DNA synthesis (Villani et al., 1978). Such an enzyme might have a low affinity for undamaged DNA or be present at too low a concentration to be detectable in a system using a non-irradiated DNA template. To test this, the polymerizing activities of extracts of u.v.-induced R⁻ and R⁺ polA⁺ and polA...
Absence of R plasmid DNA polymerases

Fig. 3. Incorporation of [³H]dTTP into acid-insoluble material by cell extracts of u.v.-induced E. coli MM300 (polA⁺) strains [R⁻ (○), R205 (□), R46 (●)] measured using DNA template that had been u.v.-irradiated at 2.8 J m⁻² s⁻¹ for different periods. Reaction mixture no. 1 was used.

Fig. 4. Incorporation of [³H]dTTP into acid-insoluble material by cell extracts of u.v.-induced E. coli AB1157 (polA⁺) strains [R⁻ (○), R205 (△), R46 (■, □)] measured using unirradiated DNA template (closed symbols) and DNA template that had been u.v.-irradiated for 25 min at 2.8 J m⁻² s⁻¹ (open symbols). Reaction mixture no. 1 was used.

strains on activated DNA templates that had been u.v.-irradiated for various times were measured. The reduction in polymerization produced by u.v. irradiation of the DNA template (Fig. 3) was similar to that found by Bowerstock & Moses (1973) for the inhibition of chromosome replication after u.v. irradiation of E. coli permeabilized by toluene-treatment. This suggests that pyrimidine dimers are indeed blocking the progress of DNA polymerase I along template DNA resulting in a reduction in the total amount of DNA synthesized. However, the presence of plasmids R205 or R46 in the polA⁺ E. coli strain did not increase DNA synthesis on u.v.-irradiated templates above that found in extracts of the R⁻ strain (Fig. 3). Similarly, the inclusion of an irradiated template failed to reveal any polymerizing activity in extracts of either the R⁺ or R⁻ polA E. coli strains (results not shown). These results indicate that the plasmids neither code for a DNA polymerase with activity specific for u.v.-damaged DNA nor alter the host DNA polymerase I such that it has an increased activity on u.v.-damaged DNA.

Plasmids R205 and R46 were also introduced into E. coli AB1157, another polA⁺ strain, and DNA polymerizing activity was measured in extracts of u.v.-induced cultures using irradiated and non-irradiated DNA templates (Fig. 4). Once again, the presence of R205 and R46 did not alter the level of DNA polymerization from that of the R⁻ strain on either irradiated or unirradiated activated DNA templates.

DISCUSSION

Kronish & Walker (1979) failed to detect a DNA polymerase I-like activity associated with the u.v.-protecting plasmids R205 and pKM101 in polA⁺ or polA E. coli strains. We have also been unable to detect any such activity associated with the three plasmids R205, R46 or pKM101 (Fig. 1). When cell extracts of u.v.-induced cultures were tested, we were again unable to detect any plasmid-associated DNA polymerase I-like activity (Fig. 2). Furthermore, even with the inclusion of u.v.-damaged DNA in the reaction mixture as template for polymerization, neither a plasmid-coded DNA polymerase I-like activity nor a
plasmid-associated increase in activity of the host chromosomal DNA polymerase I was observed, even though plasmids R205 and R46 were tested in two polA+ E. coli strains (Figs 3 and 4).

Although MacPhee (1974) reported the presence of a DNA polymerase I-like activity associated with R205 in Salmonella typhimurium, the results of Kronish & Walker (1979) and those presented in this paper suggest that the u.v.-protecting plasmids R205, R46 and pKM101 do not code for the synthesis of a novel DNA polymerase I-like enzyme in E. coli either constitutively or through a u.v.-inducible pathway. To explain these apparently conflicting results Kronish & Walker (1979) proposed that the DNA polymerization described by MacPhee (1974) might not be due to a plasmid-coded enzyme but that the plasmids R205 and pKM101 might code for a protein that interacts with the host DNA polymerase I to increase survival and mutagenesis after u.v. irradiation. Thus, a plasmid-associated DNA polymerase activity would only be observed in polA strains if this plasmid-coded protein were able to stabilize and phenotypically suppress the mutant polA enzyme. However, Kronish & Walker (1979) using plasmids R205 and pKM101 in several E. coli strains with different polA alleles were unable to detect any such plasmid-associated DNA polymerase activity. We have shown that plasmids R205, R46 and pKM101 protect the host from u.v. light and also increase u.v.-induced mutagenesis in E. coli polA strain JG138 even though no plasmid-associated DNA polymerase I-like activity could be detected in this strain. It is therefore likely that these u.v.-protecting plasmids do not code for a DNA polymerase I-like enzyme or, indeed, modify the host DNA polymerase I in E. coli. An alternative explanation of this u.v.-protecting and mutagenic activity is that such plasmids code for one or more proteins that modify host DNA polymerases I or II so as to increase their capacity for mutagenic DNA repair. It is also possible that such a modified DNA polymerase is only detectable as a DNA polymerase I-like activity in certain species of bacteria, which would explain the differences found with plasmid R205 in S. typhimurium and E. coli.

REFERENCES


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Absence of R plasmid DNA polymerases


