SHORT COMMUNICATION

Role of DNA Repair Genes and an R Plasmid in Conferring Cryoresistance on Pseudomonas aeruginosa

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The resistance of Pseudomonas aeruginosa wild-type, uvr, pol and rec strains to ultraviolet (u.v.) light, X-rays and freezing and thawing was determined. An R plasmid, pPL1, which increased resistance of the wild-type, uvr, and pol but not rec strains to u.v. light, increased the resistance of only rec and pol mutants to X-rays and freezing and thawing. These findings reinforce the idea of DNA as a target in the organism for freeze–thaw stress and suggest that freeze–thaw-induced DNA damage might be similar to that produced by X-rays but different from that produced by u.v. light.

INTRODUCTION

In bacteria, the cell membrane, wall and metabolic machinery are particularly susceptible to the effects of freezing and thawing (Mazur, 1970; Ray & Speck, 1973; MacLeod & Calcott, 1976; Lee et al., 1977; Beuchat, 1978; Calcott, 1978; Calcott et al., 1979a; Ghani & Calcott, 1980). In addition, single-stranded breaks can be detected in the DNA of Escherichia coli after freezing and thawing (Swartz, 1970, 1971a; Alur & Grecz, 1975; Alur et al., 1977; Song & Calcott, 1980) and cryoprotectants which prevent loss of viability also protect the DNA from damage (Alur et al., 1977; Song & Calcott, 1980). Free radicals are produced in frozen bacteria and could play a role in damaging the macromolecule (Cox & Heckly, 1973). These studies suggest that DNA might be the prime target for the stress of freezing and thawing (Swartz, 1971a, b; Cox & Heckly, 1973; Alur & Grecz, 1975; Alur et al., 1977; Song & Calcott, 1980; Williams & Calcott, 1980; Calcott & Gargett, 1981), although other workers believe that DNA is immune to damage from freeze–thaw (Ashwood-Smith, 1965; Ashwood-Smith et al., 1972; Ashwood-Smith & Grant, 1976).

Not only can DNA be damaged by freeze–thaw stress under certain conditions but the process can be mutagenic (Postgate & Hunter, 1961; Calcott & Gargett, 1981). The mutagenicity of freeze–thaw is also disputed by some workers (Ashwood-Smith, 1965; Ashwood-Smith & Grant, 1976). However, Calcott & Gargett (1981) believe that Ashwood-Smith’s failure to detect DNA damage and mutation in frozen–thawed bacteria was an operational artefact.

This led us to question whether DNA damage which occurs on freezing and thawing is lethal to a bacterium. In this paper, we have examined the role of two major DNA repair pathways, excision repair (uvr-, pol-dependent) and recombinational repair (rec-, pol-dependent) in the repair of DNA damage (Bridges, 1976) by determining the sensitivity of three isogenic mutants of Pseudomonas aeruginosa, and derivatives of each containing the plasmid pPL1, to freezing and thawing, ultraviolet (u.v.) light and X-rays. The genetically characterized plasmid pPL1 confers resistance to u.v. light on P. aeruginosa.

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METHODS

Organisms and cultural conditions. Pseudomonas aeruginosa PAO1 and three isogenic mutants, PAO2003 (rec-2), GMA918 (uvr-81), MMS19 (pol-2) and each of these carrying the plasmid pPL1 (Lehrbach et al., 1977a, b) were grown aerobically to late exponential/early stationary phase at 37 °C in nutrient broth. Cultures were harvested, washed and resuspended in distilled water or 0.85% saline (Calcott et al., 1979a). Pseudomonas aeruginosa MMS19(pPL1) was constructed by generalized transduction of the plasmid into MMS19 with bacteriophage E79. All strains were the generous gift of Philip Lehrbach, University of Melbourne, Victoria, Australia.

Stressing regimes. Washed suspensions of organisms were irradiated with a short wavelength u.v. light (Guth Lighting Company) or an X-ray source (Faxitron model 4380N; Hewlett-Packard, Palo Alto, Calif., U.S.A.), of power outputs 20 J m⁻² s⁻¹ and 480 rad min⁻¹, respectively. Suspensions were frozen at 1–2 °C min⁻¹ (slow cooling) or 100 °C min⁻¹ (rapid cooling). After at least 5 min at –196 °C, samples were thawed by rapid warming (100 °C min⁻¹) as described before (Calcott et al., 1979b).

Viability determination. Viable counts of stressed and unstressed populations were made by surface plating portions of suitably diluted preparations and incubating the plates at 37 °C. Viabilities were determined with reference to the viable counts of unstressed populations as described before (Calcott et al., 1979a). A dose to produce a 90% killing of the population (LD₉₀) was determined from plots of logarithm of viability versus dose, and is expressed in J m⁻², rads and cycles for u.v. light, X-rays and freeze–thaw respectively.

RESULTS

Mutant strains (rec, uvr and pol) of P. aeruginosa were more susceptible to u.v. light than the wild-type strain (Table 1). This was in agreement with the findings of Lehrbach et al. (1977a, b, 1978). The rec and pol strains were more sensitive to X-rays than the wild-type strain (Table 1) while the uvr mutant was slightly more resistant. In general, freezing in saline was more lethal to each strain than freezing in water, when the populations were cooled rapidly or slowly (Table 1). The rec and pol strains were extremely sensitive to freeze–thaw in both liquids when compared with the wild-type strain; the uvr strain was slightly more sensitive than the wild-type (Table 1).

Introduction of the plasmid pPL1 into the strains altered their sensitivities to u.v. light, X-rays and freezing and thawing (Table 1). Wild-type, uvr and pol strains carrying pPL1 became more resistant to u.v. light, while the rec(pPL1) strain was as sensitive as the plasmid-free strain. Introduction of the plasmid into the rec and pol strains but not the wild-type or uvr strains, increased their resistance to X-rays and also to freezing and thawing in water or saline (Table 1).

Table 1. Effect of plasmid pPL1 on the sensitivity of Pseudomonas aeruginosa strains to u.v.- and X-irradiation and freezing and thawing

<table>
<thead>
<tr>
<th>Strain*</th>
<th>DNA repair characteristics</th>
<th>U.v. (J m⁻²)</th>
<th>X-rays (rad)</th>
<th>Rapid freeze–thaw (cycles) in:</th>
<th>Slow freeze–thaw (cycles) in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Water</td>
<td>Saline</td>
<td>Water</td>
</tr>
<tr>
<td>PAO1</td>
<td>Wild-type</td>
<td>600</td>
<td>3840</td>
<td>4.0</td>
<td>1.5</td>
</tr>
<tr>
<td>PAO1(pPL1)</td>
<td>Wild-type + plasmid gene(s)</td>
<td>1600</td>
<td>4000</td>
<td>3.5</td>
<td>1.5</td>
</tr>
<tr>
<td>PAO2003</td>
<td>rec</td>
<td>120</td>
<td>1920</td>
<td>0.67</td>
<td>0.33</td>
</tr>
<tr>
<td>PAO2003(pPL1)</td>
<td>rec + plasmid gene(s)</td>
<td>130</td>
<td>4800</td>
<td>2.5</td>
<td>1.0</td>
</tr>
<tr>
<td>GMA918</td>
<td>uvr</td>
<td>40</td>
<td>5460</td>
<td>3.0</td>
<td>1.0</td>
</tr>
<tr>
<td>GMA918(pPL1)</td>
<td>uvr + plasmid gene(s)</td>
<td>140</td>
<td>4800</td>
<td>3.2</td>
<td>0.95</td>
</tr>
<tr>
<td>MMS19</td>
<td>pol</td>
<td>160</td>
<td>2400</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>MMS19(pPL1)</td>
<td>pol + plasmid gene(s)</td>
<td>700</td>
<td>7200</td>
<td>2.5</td>
<td>2.0</td>
</tr>
</tbody>
</table>

* Strains and their genetics are described by Lehrbach et al. (1977b).

ND, Not determined.
DISCUSSION

The response of the wild-type and three isogenic strains of *Pseudomonas aeruginosa* to u.v. light was in agreement with that reported by Lehrbach *et al.* (1977a, b, 1978, 1979). In addition, the requirement for a rec\(^+\) genotype for plasmid-mediated resistance to u.v. light was confirmed (Lehrbach *et al.*, 1977a, b, 1978, 1979). Lehrbach *et al.* (1977a, b, 1978, 1979) also concluded that an intact rec gene was required to confer resistance to \(\gamma\)-rays and certain mutagens, and to increase u.v. light-induced mutagenesis. These have not been tested in this study. However, we have determined the role of the various genes in plasmid-mediated resistance to X-rays. The patterns were different from those found for u.v. light; an intact rec or pol gene was not required. It is apparent that repair of X-ray-induced damage can be accomplished in the absence of a functional rec or pol gene by replacement with the plasmid gene(s). However, in wild-type or uvr strains, the rec and pol gene products were not limiting since the plasmid gene product(s) could not augment their functions. Similarly, the rec- and pol-dependent steps could be replaced by the plasmid gene product(s) to repair freeze–thaw-induced DNA damage in rec and pol but not wild-type or uvr strains. Plasmid pP2L (isogenic to pP1L), which has lost the ability to protect *P. aeruginosa* from u.v. light and \(\gamma\)-rays (Lehrbach *et al.*, 1977a, b, 1978, 1979), was assessed for ability to protect the organism from X-ray and freeze–thaw sensitivity. This plasmid failed to protect the rec strain from X-rays or fast freeze–thaw in water or saline (results not presented), indicating that the gene responsible for protection of organisms against u.v. light also protected against X-ray and freeze–thaw damage. This study also indicated that the damage introduced by u.v. light, \(\gamma\)-rays and certain mutagens was repaired differently from that introduced by X-rays or freeze–thaw.

This study supports the concept that the DNA of bacteria could be a target for the stress of freezing and thawing, with rec- and pol-dependent pathways (most probably the error-prone, recombinational repair pathway) playing a major role in repair of this type of DNA damage and the uvr-dependent pathway (most probably the excision repair pathway) playing a minor role. The evidence for the involvement of the recombinational repair pathway is strengthened by the fact that freezing and thawing is mutagenic to *Escherichia coli* wild-type and uvrA, but not recA strains (Calcott & Gargett, 1981). Other studies have also indicated that other stresses such as mild heating (Bridges *et al.*, 1969; Sedgwick & Bridges, 1972), freeze-drying (Ashwood-Smith *et al.*, 1972) and cold shock (Sato & Takahashi, 1970) can cause DNA damage and/or mutation.

The pattern of response of the mutants to the plasmid was similar for the stresses of X-ray and freeze–thaw. This would indicate that the types of damage and the mechanism of repair might be similar for the two stresses. X-rays produce single and double-stranded DNA breaks and base alterations (see Bridges, 1976), while freezing and thawing introduces single-strand and possibly double-strand breaks (Alur & Grecz, 1975; MacLeod & Calcott, 1976; Alur *et al.*, 1977; Song & Calcott, 1980); the occurrence of base alteration has not been determined. It is not known whether the DNA damage introduced by freeze–thaw is caused by physical, chemical or enzymic mechanisms.

No difference was seen in the response of the various mutant strains and the plasmid in populations subjected to slow versus rapid freezing and thawing, for which damage has been proposed to be different (Mazur, 1970; MacLeod & Calcott, 1976; Calcott, 1978). Thus this study does not support Mazur's 'Two-Factor Hypothesis' (Mazur, 1970), which states that cells subjected to slow freezing succumb to 'solution effects' while those frozen rapidly succumb to 'ice-crystal damage'.

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REFERENCES


