Effect of Bacteriophage C5 on Ultraviolet Light Survival in *Pseudomonas aeruginosa*

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Bacteriophage C5 of *Pseudomonas aeruginosa* is able to reactivate ultraviolet (u.v.)-irradiated phage E79 in coinfection experiments and decrease the u.v.-sensitivity of a host-cell reactivation deficient mutant. These properties suggest that phage C5 has a gene(s) which is involved in the repair of u.v.-damaged DNA. The isolation of two u.v.-sensitive mutants of C5 supports this hypothesis.

INTRODUCTION

The survival of a bacteriophage following ultraviolet (u.v.) irradiation is mainly dependent on the capacity of the host cell systems that repair the u.v.-damaged DNA. Some bacteriophages, however, are able to repair u.v.-induced damage by a phage-coded enzymic dark repair system. In the case of the *Escherichia coli* bacteriophage T4, at least three bacteriophage-coded genes – ν (Harm, 1963), χ (Harm, 1963) and γ (Boyle & Symonds, 1969) – have been implicated in the repair of u.v.-damaged DNA. The ν -gene codes for an endonuclease which carries out the incision step of excision repair (Yasuda & Sekiguchi, 1970; Friedberg & King, 1971). Furthermore, Harm (1968) has shown that the ν -gene product does not discriminate between bacterial and phage DNA so that the u.v.-survival of *E. coli* strain B₈₋₁ can be significantly increased following infection with heavily irradiated T4.

In this report we describe some properties of the *Pseudomonas aeruginosa* bacteriophage C5 (Bradley & Pitt, 1974) which affect the repair of u.v. damage in bacteriophage and bacterial DNA.

METHODS

Media and general culture procedures. These techniques have been described previously (Kung & Lee, 1973). Layer agar for bacteriophage assays was Bresch layer agar containing 1 % (w/v) Bacto-tryptone, 0.5 % (w/v) yeast extract, 0.05 % (w/v) NaCl and solidified with 1.0 % (w/v) agar.

Bacterial and bacteriophage strains. The standard wild-type strain is *Pseudomonas aeruginosa* PAO1 (ATCC 15692) (Holloway, 1955). Strain GMA918 was derived from PAO1 (Kung & Lee, 1973) by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) mutagenesis and shows increased sensitivity to u.v. light and reduced ability to reactivate u.v.-treated phage E79; it is a selected member of phenotypic class 9 (Kung & Lee, 1973) and is considered to be a host-cell reactivation (*hcr*) mutant. Strain GMC161 was isolated by the method of Kropinski *et al.* (1977) as a spontaneous C5-resistant, E79-sensitive mutant of GMA918 and was used in coinfection experiments.

Bacteriophage C5 has been described by Bradley & Pitt (1974) and was obtained from G. A. Jacoby. Bacteriophage E79 has been described by Holloway *et al.* (1960).

Host-cell reactivation (*hcr*) experiments. In *hcr* experiments, a Tris/sodium/magnesium buffer (TNM) (Kung & Lee, 1973) suspension of the phage was exposed to various doses of u.v.-irradiation as previously described (Kung & Lee, 1973). The irradiated phage was then diluted and assayed on the appropriate test strains.

Recovery of u.v.-irradiated E79 by coinfection with heavily irradiated C5. An overnight (16 h at 37 °C)

Fig. 1

Fig. 2

Fig. 1. Host-cell reactivation of u.v.-irradiated phage E79 (■, ●) and phage C5 (□, ○) on strains: ■, □, GMA918; ●, ○, PAO1.

Fig. 2. Host-cell reactivation of u.v.-irradiated phage E79 on strains: ●, PAO1; ■, GMA918; △, GMA918 plus heavily u.v.-irradiated phage C5 (200 J m⁻², survival 1 in 10⁸, m.o.i. 5 to 10); ▲, GMA918 plus heavily u.v.-irradiated mutant phage C5(101) (180 J m⁻², survival 1 in 10⁸, m.o.i. 5 to 10); ▼, GMA918 plus heavily u.v.-irradiated mutant phage C5(102) (180 J m⁻², survival 1 in 10⁸, m.o.i. 5 to 10).

shaken broth culture of strain GMA918 was infected with phage E79, exposed to various doses of u.v. light, at a multiplicity of infection (m.o.i.) of 1 and coinfectd with heavily u.v.-irradiated (200 J m⁻²) bacteriophage C5 (survival 1 in 10⁸; m.o.i. 5 to 10). After 10 min incubation at 37 °C, to allow for phage adsorption, the cells were diluted and the survival of E79 was assayed on GMC161. Control experiments were performed in a similar manner, using the same suspension of phage E79 (m.o.i. 1) but no bacteriophage C5 was added.

U.v.-survival of GMA918 following infection with heavily irradiated C5. TNM buffer suspensions of GMA918 were exposed to various doses of u.v. light and immediately infected with heavily u.v.-irradiated (200 J m⁻²) bacteriophage C5 (survival 1 in 10⁸; m.o.i. 5 to 10). After 10 min incubation at 37 °C, the cells were diluted and assayed for survival on nutrient agar. Plates were incubated overnight at 37 °C before colony counts were made.

Bacteriophage mutagenesis. This procedure was used to isolate u.v.-sensitive mutants of phage C5. An overnight (16 h at 37 °C) shaken broth culture was centrifuged and resuspended in 1 ml citrate buffer (pH 5.8) and an equal volume of phage C5 (m.o.i. 1) was added. Immediately, NTG (100 µg ml⁻¹) was added and the suspension was incubated at 37 °C without shaking for 60 min. A sample was then withdrawn, diluted and plated on PAO1 to obtain C5 plaques. Individual plaques were picked and phage particles were suspended in 0.5 ml TNM buffer. Control and u.v.-irradiated phage were replica-plated on to prepared lawns of PAO1 and GMA918. Phage isolates showing increased sensitivity when plated on GMA918 but normal plating on PAO1 were further tested and characterized. Two u.v.-sensitive mutants of C5 designated by the strain numbers C5(101) and C5(102) were isolated.

RESULTS AND DISCUSSION

The survival of u.v.-irradiated phage C5 and E79 plated on host cells PAO1 (wild-type for repair) and a hcr mutant GMA918 are shown in Fig. 1. As previously described (Kung & Lee, 1973), GMA918 showed a greatly reduced ability to host-cell reactivate (hcr) u.v.-irradiated phage E79 compared with PAO1. This difference in hcr capacity was not shown for phage C5 where there was only a slight difference in the u.v.-survival curves on PAO1 and GMA918. The different u.v.-survival of phages C5 and E79 on GMA918 could be due to the

Fig. 3

Fig. 3. U.v.-survival of strain GMA918 following infection with heavily u.v.-irradiated phage: ●, C5; ▲, C5(101); ▼, C5(102); △, E79; □, no phage added.

Fig. 4

Fig. 4. Host-cell reactivation of u.v.-irradiated phage C5 (○, □), C5(101) (▼, ▲) and C5 (102) (●, ■) on strains: ○, ●, ▼, PAO1; □, ▲, ■, GMA918.

presence of phage-determined gene(s) in phage C5 which partially compensate for the repair deficiency of GMA918. To test this hypothesis, the survival of u.v.-irradiated phage E79 on both PAO1 and GMA918 as well as the u.v.-survival of GMA918 following infection with heavily u.v.-irradiated phage C5 was determined.

The survival of u.v.-irradiated phage E79 infecting GMA918 simultaneously infected with heavily u.v.-irradiated phage C5 (m.o.i. 5 to 10), the survival of u.v.-irradiated phage C5 singly infecting GMA918 and the survival of u.v.-irradiated phage E79 singly infecting GMA918 were determined (Fig. 2). The u.v.-survival of phage E79 in these experiments was determined on strain GMC161. The results show that simultaneous infection with heavily u.v.-irradiated C5 is able to increase the survival of u.v.-irradiated phage E79. Further evidence for the hypothesis that phage C5 determines a gene product(s) which may participate in the repair of u.v.-damaged DNA was obtained by determining the u.v.-survival of GMA918 infected with heavily u.v.-irradiated phage C5 (Fig. 3). Phage C5 considerably affects the survival of GMA918 at the u.v. doses tested, whereas infection with heavily u.v.-irradiated (180 J m^{-2}) phage E79 (survival $1 \text{ in } 10^8$; m.o.i. 5 to 10) had no effect on the u.v.-survival of GMA918. Therefore it seems likely that a gene product(s) determined by the phage C5 genome is able to modify the u.v.-survival of phage E79 on a *hcr* mutant and the u.v.-survival of GMA918; furthermore, such a phage function does not appear to be present in the phage E79 genome. The isolation of two u.v.-sensitive mutants of phage C5 provides further evidence that phage C5 contributes a gene product or products that participate in the repair of u.v.-damaged DNA. These mutant strains C5(101) and C5(102) showed increased u.v.-sensitivity when plated on GMA918 and to a lesser degree on PAO1 (Fig. 4). Furthermore, these mutants do not modify the u.v.-survival of u.v.-irradiated phage E79 (Fig. 2). Therefore phage C5 appears to have similar properties to the u.v.-resistant *E. coli* phage T4 which is able, under appropriate conditions, to increase the u.v.-survival of u.v.-irradiated phage T2 (Harm, 1961) and to effect the recovery of the u.v.-sensitive *E. coli* strain B₈₋₁ (Harm, 1968) following u.v.-irradiation.

Further studies will be initiated to define the mode of action of C5 gene(s) in the repair of u.v.-damaged DNA, its expression and the phage gene product(s) involved.

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