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Repair of Ultraviolet Light-induced DNA Damage in Cholera Bacteriophages

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SUMMARY

DNA repair-proficient and -deficient strains of *Vibrio cholerae* were used to examine host cell reactivation, Weigle reactivation and photoreactivation of u.v.-irradiated cholera bacteriophages. U.v. light-induced DNA damage in phages of different morphological and serological groups could be efficiently photoreactivated. Host cell reactivation of irradiated phages of different groups was different on the same indicator host. Phage ϕ149 was the most sensitive, and ϕ138 the most resistant to u.v. irradiation. While ϕ138 showed appreciable host cell reactivation, this was minimal for ϕ149. Attempts to demonstrate Weigle reactivation of u.v.-irradiated cholera phages were not successful, although u.v.-induced filamentation of host cells was observed.

INTRODUCTION

Four distinct serological (Mukherjee, 1963) and morphological (Chatterjee *et al.*, 1965) groups of phages that infect *Vibrio cholerae* cells have been described. Although these phages do not have much therapeutic value, they are extremely useful for taxonomic purposes. Phages belonging to group IV are of special interest, because of their routine use in the differentiation of classical vibrios from their biotype *V. eltor* (Mukherjee, 1978).

Studies on repair of u.v.-induced DNA damage in bacteriophages have provided considerable insight into the types of DNA repair mechanisms operative in the host cells (Day, 1981; Bernstein, 1981). Recent studies on the repair of u.v.-induced DNA damage in *V. cholerae* cells have shown that most of the strains examined so far can efficiently monomerize the pyrimidine dimers by photoreactivation, although their ability to repair the DNA damage by dark-repair mechanisms is poor (Das *et al.*, 1981). The universal host strain for cholera bacteriophages, strain 154 (Mukherjee, 1963), completely lacks the excision repair mechanism for u.v.-induced DNA damage. Furthermore, the ability of repair-proficient strains to repair DNA damage in the dark is related to the level of toxinogenicity of these organisms (Roy *et al.*, 1982*b*). A radiation-sensitive mutant of the hypertoxinogenic strain 569B of classical *V. cholerae* has been shown to be phenotypically non-toxinogenic (Das & Das, 1983).

To investigate the DNA repair mechanisms of *V. cholerae* cells in more detail, we have conducted studies on repair of u.v.-irradiated cholera phages using dark-repair-proficient and -deficient host cells. The results presented here show that u.v.-induced DNA damage in cholera phages can be host cell reactivated and photoreactivated. Attempts to demonstrate Weigle reactivation have not been successful.

METHODS

Bacterial and phage strains. Strains of *V. cholerae* used in this study are listed in Table 1. The wild-type strains were obtained from the Cholera Research Centre, Calcutta, India. The radiation-sensitive, non-toxinogenic mutant 569B$_s$ of the hypertoxinogenic strain 569B was isolated in this laboratory (Das & Das, 1983). The conditions for maintenance of these strains have been described previously (Roy *et al.*, 1982*a*). Cell cultures were stored at 28 °C on nutrient agar slants.

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Table 1. V. cholerae *strains*

Strain	Genotype/phenotype*	Other information	Reference
569B	Prototroph, Tox^+, HCR^+, PR^+	Classical biotype, serotype Inaba	Finkelstein (1973)
154	Prototroph, HCR^-, PR^+	Classical biotype, serotype Ogawa	Mukherjee (1978)
$569B_s$	Prototroph, Tox^-, HCR^-, PR^+	Radiation-sensitive mutant of 569B	Das & Das (1983)

* Tox^+, Toxinogenic; HCR^+, able to perform host cell reactivation; PR^+, able to photoreactivate.

Table 2. *Cholera bacteriophages*

				Infectivity	
Phage*	Classification†	Nature of* infection	Nucleic acid‡	*V. cholerae*	*V. eltor*
ϕ163	Group I	Virulent	DNA	+	+
ϕ138	Group II	Virulent	Double-stranded DNA	+	+
ϕ145	Group III	Virulent	DNA	+	+
ϕ149	Group IV	Virulent	Double-stranded linear DNA, mol. wt. 96×10^6	+	−

* Mukherjee (1963).
† Classification based on serological (Mukherjee, 1963) and morphological results (Chatterjee *et al.*, 1965).
‡ A. Sengupta, P. Roy & J. Das, unpublished observations.

One representative strain of each of the four groups of cholera bacteriophages described in Table 2 was used in the present study.

Growth media and buffer. The nutrient broth used contained 10 g Bactopeptone (Difco), 10 g Lab-Lemco powder (Oxoid) and 5 g NaCl dissolved in 1 litre distilled water; the pH was 7·8. Nutrient agar plates contained 1·5% (w/v) Bactoagar (Difco) in nutrient broth. The soft agar for double-layer plating was 0·75% (w/v) Bactoagar in nutrient broth. Cell and phage viabilities were assayed on nutrient agar plates as colony-forming units (c.f.u.) and plaque-forming units (p.f.u.) respectively.

The holding buffer used was 50 mM-Tris–HCl pH 7·5 containing 5 mM-$MgCl_2$.

Preparation of phage stock. Phages were seeded on lawns of host bacteria on nutrient agar plates and incubated at 37 °C overnight to obtain confluent lysis as described previously (Balganesh & Das, 1979). Phages were eluted from the plates by adding about 5 ml of holding buffer and centrifuged (10000 *g* for 20 min) to remove cell debris. The supernatant was then centrifuged in a Sorvall A841 rotor at 35000 rev/min for 1 h using a Sorvall OTD 50 ultracentrifuge. The phage pellet was resuspended in holding buffer.

Irradiation conditions. For u.v. irradiation, phage suspensions (1×10^9 to 5×10^9 p.f.u./ml) in holding buffer were irradiated in an 80 mm diam. Petri dish at room temperature (28 °C) in the dark with constant agitation, using a 15 W Philips germicidal lamp emitting primarily at 254 nm at a dose rate of 1 $J/m^2/s$. At different doses, 0·1 ml samples were removed and assayed for p.f.u.

Photoreactivation (PR). Irradiated phages were mixed with host cells in soft agar and plated on nutrient agar. The plates were exposed to visible light (40 W fluorescent lamp at a distance of 12 to 13 cm). At different times during this irradiation, plates were removed and incubated in the dark.

Host cell reactivation (HCR). This was demonstrated by assaying survival of u.v.-irradiated phages using repair-proficient and -deficient indicator cells.

Weigle reactivation (WR). Irradiated phages were mixed in holding buffer with either unirradiated cells or cells irradiated with different doses of u.v.; the multiplicity of infection was about 0·05. Ten min was allowed for adsorption at room temperature and then the phages were titrated.

RESULTS AND DISCUSSION

Sensitivity of cholera phages to u.v. light

One representative strain of each of the four groups of cholera phages was examined for its sensitivity to u.v. light. Survival as a function of u.v. dose for all four phages when plated on the universal host strain 154 was exponential, indicating one-hit kinetics (Fig. 1). A comparison of the inactivation cross-sections showed that ϕ138 (inactivation cross-section 0·017 m^2/J) was the most resistant; ϕ149 (inactivation cross-section 0·09 m^2/J) and ϕ163 (data not shown) were the most sensitive to u.v. light. ϕ145 (inactivation cross-section 0·07 m^2/J) showed intermediate sensitivity. ϕ149 and ϕ163 could not be distinguished by their u.v. sensitivity. These latter two phages were checked for cross-contamination, and found to be pure from phage morphology,

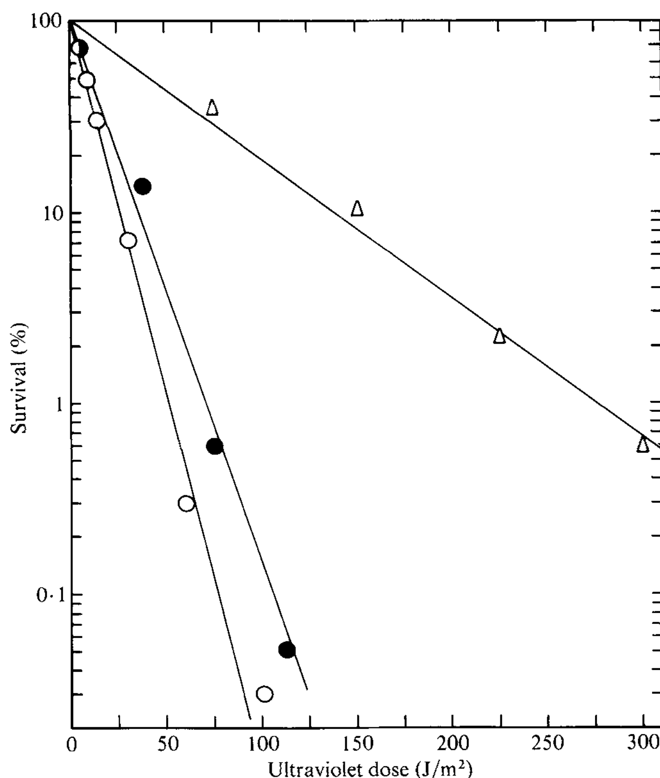

Fig. 1. Survival of u.v.-irradiated cholera bacteriophages using strain 154 as the indicator host. Phages ϕ149 (○), ϕ145 (●) and ϕ138 (△) were irradiated as described in Methods. Each point in this graph represents an average from three to five experiments.

serology and infectivity on *V. eltor* cells. Whereas ϕ163 can infect both classical vibrios and their biotype, *V. eltor*, ϕ149 cannot infect *V. eltor* cells. In subsequent experiments the repair of u.v.-induced DNA damage in irradiated ϕ138 and ϕ149 was examined.

HCR of u.v.-irradiated phages

HCR is the repair of damage in phage DNA brought about by removal of pyrimidine dimers by the host cell excision repair mechanism and presumably does not involve any viral gene function (Day, 1981; Bernstein, 1981). The sensitivity of u.v.-irradiated phages therefore depends upon the genotype and phenotype of the host used for assay [it is possible to produce HCR^- phenocopies of HCR^+ cells (Feiner & Hill, 1963; Das *et al.*, 1977)].

To examine whether u.v.-irradiated ϕ138 or ϕ149 can be host cell-reactivated, they were irradiated with different doses of u.v. light and survival was assayed using excision-proficient cells of strain 569B and excision-deficient cells of strain 154 as indicator hosts. In some experiments, a radiation-sensitive mutant, $569B_s$, of the strain 569B was used as host. The efficiencies of plating of ϕ138 and ϕ149 on these indicator strains were almost identical.

A two fold reactivation of u.v.-irradiated ϕ138 was observed when cells of strains 154 and 569B were used (Fig. 2*a*). However, when $569B_s$ cells were used for assay, the sensitivity of irradiated ϕ138 increased seven- to eightfold relative to that on cells of strains 154 or 569B (Fig. 2*a*). Although $569B_s$ and 154 cells both lack an excision repair mechanism, the former is also deficient in the growth medium-dependent dark recovery process (Das & Das, 1983) which, in *Escherichia coli*, involves recombination functions. It has been shown that even in the presence of a functional *uvrA* gene product only 15% of the infected cells can host cell-reactivate irradiated phages if the host *recA* gene is non-functional (Day, 1981). The enhanced sensitivity

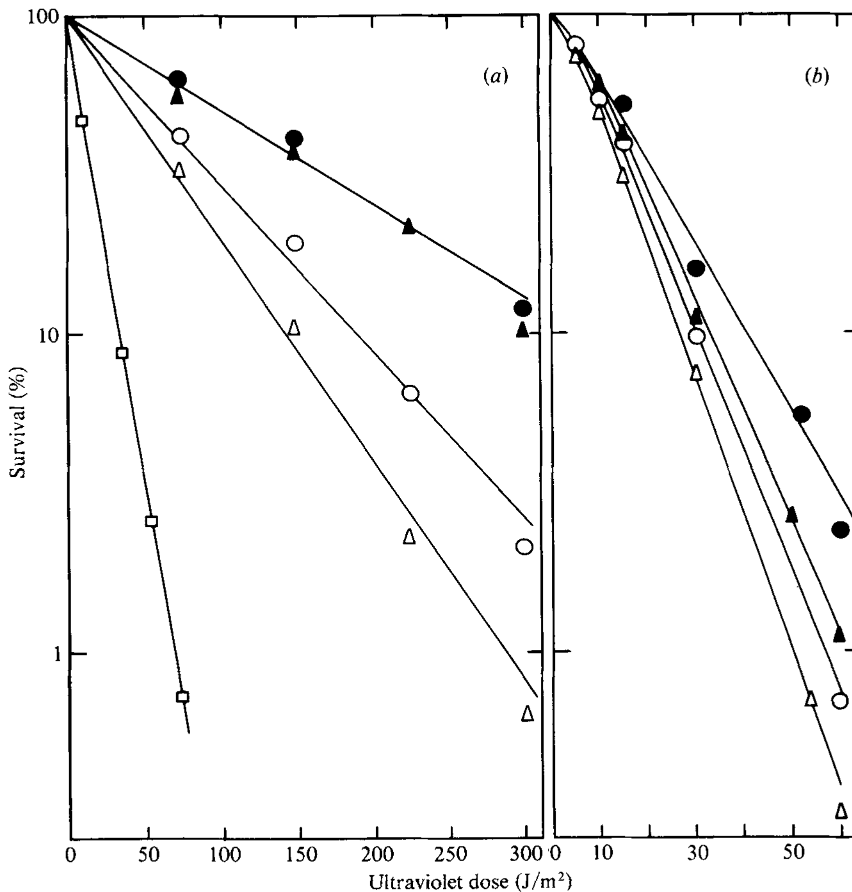

Fig. 2. Host cell reactivation and photoreactivation of u.v.-irradiated (*a*) phage ϕ138 on strains 154 (△, ▲), 569B (○, ●) and 569B$_s$ (□) and (*b*) phage ϕ149 on strains 154 (△, ▲) and 569B (○, ●) as hosts. At each u.v. dose two samples were removed. One was immediately plated to determine the u.v. survival curve (△, ○, □) and the second sample was plated and exposed to visible light for 3 h, then incubated at 37 °C in the dark (▲, ●).

of irradiated ϕ138 when assayed on cells of strain 569B$_s$ suggests a defect in the recombination function of the mutant cells.

Not more than a 1·5-fold difference in u.v. sensitivity of ϕ149 was observed when cells of strains 154 and 569B were used to assay its survival (Fig. 2*b*). An identical difference in sensitivity of u.v.-irradiated ϕ149 has recently been reported with cells of strains 569B and 569B$_s$ as hosts (Das & Das, 1983). The less efficient HCR of u.v.-irradiated ϕ149 compared to that of ϕ138 on the same indicator hosts might be due to the phage DNA conformation. It has recently been shown that ϕ149 contains a linear double-stranded DNA having a mol. wt. of 96 × 10^6 (140 kilobase pairs) which can be cleaved at a unique site by S1 nuclease. The S1-sensitive site represents a nick in the DNA; it can be repaired by T4 DNA ligase (A. Sengupta, P. Roy & J. Das, unpublished observation). Whether the presence of the nick in ϕ149 DNA has any effect on reactivation of irradiated phage is not clear. Interestingly, *E. coli* phage T5 DNA also exhibits single-strand interruptions at several genetically defined loci and also can not be host cell-reactivated (Abelson & Thomas, 1966; Chiang & Harm, 1976).

HCR of u.v.-irradiated cholera phages was low compared to that of *E. coli* phages. This is not surprising considering that 569B cells are not proficient in this mode of repair (Das *et al.*, 1981; Roy *et al.*, 1982*b*), although they can repair u.v.-induced DNA damage in the dark more efficiently than other strains of *V. cholerae* examined so far.

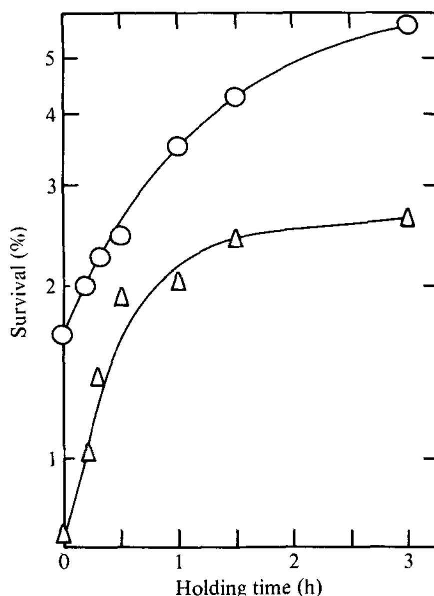

Fig. 3. Photoreactivation kinetics of u.v.-irradiated ϕ149 using cells of strains 569B (○) or 154 (△) as indicator hosts.

PR of u.v.-irradiated cholera phages

PR involves visible light-dependent enzymic monomerization of u.v.-induced pyrimidine dimers. Except for the T4 gene *32* product, which can monomerize thymidine dimers in the presence of visible light *in vitro* (Hélène *et al.*, 1976), photoreactivation of u.v.-irradiated bacteriophages utilizes the host cell photoreactivating enzyme (Dulbecco, 1949; Bowen, 1953; Weigle, 1953). To examine whether cholera phages can be photoreactivated, cells of strains 569B or 154 were infected with irradiated ϕ138 or ϕ149 and then were exposed to visible light as described in Methods. The results presented here show that u.v.-induced DNA damage in both ϕ138 (Fig. 2*a*) and ϕ149 (Fig. 2*b* and 3) could be repaired by photoreactivation. Irradiated ϕ138 could also be photoreactivated when the dark-repair-deficient host $569B_s$ was used for assay (data not shown). The extent of reactivation of irradiated ϕ149 by either host was almost identical (Fig. 2*b* and 3). However, in contrast to the approximately ninefold reactivation of u.v.-irradiated ϕ138 on 154 cells, a recovery of not more than fivefold was observed when cells of strain 569B were used to assay the phage survival (Fig. 2*a, b*). Since the PR observed is the result of competition between dark- and light-repair processes acting on the same lesion, this host strain dependence of the extent of PR of irradiated ϕ138 might be due to HCR in cells of strain 569B.

WR of u.v.-irradiated cholera phages

WR is observed as an increased survival of u.v.-irradiated phages when assayed on a host that has also been lightly irradiated with u.v. before the infection (Weigle, 1953). In contrast to the error-free nature of most repair mechanisms, WR is characterized as an error-prone process accompanied by mutation (Weigle, 1953; Radman, 1974) and involves a u.v.-induced repair system distinct from both the excision repair pathway responsible for HCR (Boyle & Setlow, 1970) and PR by photoreactivating enzymes. It is also distinct from recombinational repair (Ganesan, 1975; Witkin, 1976). WR has been demonstrated even in u.v.-irradiated single-stranded DNA phages (Tessman & Ozaki, 1960; Bleichrodt & Verheij, 1974; Das *et al.*, 1977).

To find out whether this mode of repair is operative in *V. cholerae* cells, it was investigated using ϕ138 and ϕ149, by comparing the survival of u.v.-irradiated phages on unirradiated cells and on cells irradiated with u.v. prior to infection. Both 569B and 154 cells were used and

possible reactivation was examined at several u.v. doses (up to 25 J/m^2) to the cells. Under no circumstances could the irradiated phages (surviving fractions: ϕ138, approx. 6×10^{-4}; ϕ149, approx. 10^{-2}) be repaired by WR.

U.v.-induced filamentation of V. cholerae *cells*

According to the 'SOS' hypothesis (Radman, 1974; Witkin, 1976), Weigle reactivation, prophage induction, u.v.-induced mutagenesis, filamentation during cell growth and various other post-irradiation phenomena are coordinated by inducible cellular repair processes. The absence of WR of u.v.-irradiated cholera phages led us to examine whether any other of the so-called SOS functions could be demonstrated in *V. cholerae* cells.

When cells of strain 569B were irradiated with a dose of 5 J/m^2 of u.v. light (cell survival about 50%) and allowed to grow in the growth medium for 50 min (about 1·5 generation times) at 37 °C in the dark, more than 80% of the cells formed filaments. This effect was inhibited by adding 5 µg/ml chloramphenicol after u.v. irradiation. Unirradiated cells under identical conditions maintained their normal morphology. It may be relevant to mention that *umuC* mutants of *E. coli* K12, although unable to Weigle-reactivate u.v.-irradiated λ phage, are nevertheless capable of inducing other SOS functions (Kato & Shinoura, 1977; Hall & Mount, 1981).

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