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Evidence for a Near UV-Induced Photoproduct of 5-Hydroxymethylcytosine in Bacteriophage T4 That Can Be Recognized by Endonuclease V *

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Summary. Non-photoreactivable endonuclease V-sensitive sites have been detected in the DNA of wild type bacteriophage T4 irradiated with near UV light (320 nm). Such sites were not detected in the DNA of (a) wild type T4 irradiated with far UV (254 nm) or (b) in T4 mutants in which non-glucosylated 5-hydroxy-methylcytosine (5HMC) or cytosine replaces glucosylated 5HMC normally present in T4, irradiated with 320 nm or 254 nm light. Although the non-photoreactivable sites accounted for ~50% of the endonuclease V-sensitive sites in the DNA of glucosylated T4 irradiated with near UV, there was very little difference in the sensitivities of T4 containing glucosylated 5HMC, non-glucosylated 5HMC and cytosine to near UV (313 nm). We propose that the photoproduct responsible for the non-photoreactivable, but endonuclease V-sensitive, sites in glucosylated DNA is formed from glucosylated 5HMC and that a similar photoproduct is formed from non-glucosylated 5HMC or cytosine in the appropriate phage strains. We further propose that the glucosylated 5HMC photoproduct is non-photoreactivable whereas the cytosine and non-glucosylated 5HMC photoproducts are photoreactivable and are therefore possibly cyclobutane dimers.

Introduction

When DNA is irradiated with UV light a variety of photoproducts are formed [for recent reviews see Wang (1976)]. The most important of these are pyrimidine dimers, which are probably responsible for most of the lethal and mutagenic effects of far UV light (<280 nm) in bacteria and bacteriophage (Setlow, 1966, 1968; Meistrich, 1972; Meistrich and Drake, 1972). Other photoproducts, however, are implicated in lethality caused by near UV-irradiation; pyrimidine dimers do not appear to contribute significantly to 365 nm UV-induced lethality in bacteria and bacteriophage (Webb et al., 1976; Eisenstark and Ananthaswamy, 1976; Cabrera-Juárez and Setlow, 1977) and there is evidence that inactivation of bacteriophage T4 irradiated at 300 nm is due to both pyrimidine dimers and another unidentified photoproduct (Winkler et al., 1962; Cavilla and Johns, 1964). This unidentified 300 nm-induced photoproduct is of interest because, unlike other lethal near UV photoproducts formed in the DNA of *E. coli* and bacteriophage T4 (Eisenstark and Ananthaswamy, 1976; Webb and Brown, 1976; Webb et al., 1976), it can be repaired efficiently by the same excision repair pathway that is responsible for the repair of pyrimidine dimers. Evidence for this comes from studies with a UV-sensitive T4 mutant, *denV1* [formerly v_1 (Wood and Revel, 1976)] which is unable to excise pyrimidine dimers from its DNA in a wild type host (Setlow and Carrier, 1966, 1968). The first step in the excision repair pathway of pyrimidine dimers is the formation of a single strand incision near a dimer. In bacteriophage T4 infected cells this is catalyzed by endonuclease V, the product of the T4 *denV* gene (Friedberg and King, 1969; Yasuda and Sekiguchi, 1970). It has been found that the UV sensitivity of *denV1* relative to wild type increases from about 2.0 to 2.5-fold for 225 to 285 nm irradiation up to 4 to 6-fold for 302 to 305 nm irradiation (Winkler et al., 1962; Cavilla and Johns, 1964). This increase in relative UV sensitivity was correlated with a decrease in the photoreactivable sector for *denV1* (Cavilla and Johns, 1964). These results suggest that a photoproduct is formed only

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during near UV irradiation of T4 DNA that can be recognized by endonuclease V but is not photoreactivable.

In this report we present direct biochemical evidence for the presence of such a photoproduct. We have found non-photoreactivable endonuclease V-sensitive sites in DNA extracted from 320 nm-irradiated phage but not in DNA extracted from 254 nm-irradiated phage.

Materials and Methods

Media and Buffers. The various media and buffers employed for the culture of *E. coli* and T4 have been described previously (Childs, 1977). DNA dialysis buffer contained 0.05 M Na_2HPO_4 and 1 mM Na_2EDTA, adjusted to pH 7.0 with HCl.

Bacteria and Bacteriophage. Most of the bacteria and bacteriophage used have been described previously (Childs, 1977). Additional strains and their relevant characteristics are described in Table 1 and below. The nomenclature for the UV sensitive T4 mutants follows that suggested by Wood and Revel (1976); the *v* gene is now the *denV* gene (DNA endonuclease V) and the *x* gene is now the *uvsX* gene. The *galU56* mutation was introduced into *E. coli* B834 (Table 1) by Runnels and Snyder (1978) in order to provide a selective host for T4 particles containing cytosine instead of 5-hydroxymethylcytosine (5HMC); normal T4 fails to form plaques on this strain as the *E. coli galU* mutation prevents glucosylation of the 5HMC of T4 DNA (Hattman and Fukasawa, 1963; Shedlovsky and Brenner, 1963) thus exposing the 5HMC-containing DNA to the *E. coli rgl* restriction system (Revel, 1967). Cytosine-containing T4 DNA is not sensitive to this restriction system.

The T4 mutant (*denValc*) lacking a functional endonuclease V gene and containing cytosine in its DNA was obtained by first plating progeny from a cross of *denV* and *alc* on B834 *galU56* to select for cytosine-containing phage, and then testing for UV sensitivity.

The preparation of bacterial cultures and non-radioactive phage stocks has been described previously (Childs, 1977). Phage stocks labelled with radioactive thymidine were prepared by a lysis inhibition method (Doermann et al., 1970) using the following hosts: *E. coli* B for normal T4, K803 for T4 containing non-glucosylated 5HMC, and B834 for cytosine containing T4. Immediately before infection the following were added to the culture (final concentration): deoxyadenosine (250 μg/ml) and either [2-^{14}C]thymidine (0.5 μCi/ml, 53 mCi/mmol) or [^{3}Hmethyl] thymidine (10 μCi/ml, 46 Ci/mmol). Phage were purified by 2 cycles of differential centrifugation (8,000 rpm for 1 h followed by 5,000 rpm for 10 min in a Sorvall SS-34 rotor at 20° C) and resuspended in phage buffer. The same method was used to prepare radioactively labelled T4 phage containing cytosine in its DNA except that the following additions (final concentration) were made $2^1/_2$ h before infection: deoxyadenosine (250 μg/ml); thymine (100 μg/ml) and [2-^{14}C]cytosine sulphate (0.17 μCi/ml, 61 mCi/mmol). All radiochemicals were obtained from Amersham/Searle Corporation.

Determination of the Cytosine Content of alc *Mutants.* DNA was extracted from ^{14}C-cytosine labelled T4 phage with phenol as described below and hydrolyzed with 88% formic acid at 185° C for 30 min. The hydrolysate was mixed with ^{3}H-cytosine as a marker and applied to Whatman 3MM paper. Separation by paper chromatography was carried out using a solvent described by Unrau et al. (1972) consisting of (parts by volume) *n*-butanol (100), isobutyric acid (37.5), water saturated with sodium tetraborate pH 5.5 (25), 25% ammonia (2.5). In this system the R_F values for thymine, cytosine and hydroxymethylcytosine were 0.52, 0.42 and 0.31 respectively. Both *denValc* and the parental *alc* mutant had at least 95% substitution of cytosine for hydroxymethylcytosine.

Table 1. Bacterial and bacteriophage strains used

Strain	Relevant characteristics	Source	Reference
E. coli			
B834	$r_B^- m_B^- su^-$	L. Snyder	Wood (1966)
B834 *galU56*	permissive for cytosine containing but not for 5 HMC containing T4 particles	L. Snyder	Runnels and Snyder (1978)
H514	*endoI uvrA*	B. Bachmann	Vosberg and Hoffmann-Berling (1971)
K803	$r_K^- m_K^- suII^+$, permissive for non-glucosylated T4 phage	L. Snyder and E. Kutter	Wood (1966)
T4			
denV1	unable to induce endonuclease V	N. Symonds	Harm (1963)
αgt βgt	contains non-glucosylated DNA	L. Snyder	Georgopoulos (1967)
alc	*alc8 amE51* (gene 56) *denA* (*nd28*) *denB rIIH23* contains cytosine when grown on a su^- host	L. Snyder	Snyder et al. (1976)
denV1alc	UV sensitive, contains cytosine when grown on a su^- host	This study	
denV110	unable to induce endonuclease V		Childs (in preparation)
uvsX102	UV sensitive mutant with a defect in a different pathway of repair to that of *denV*		Childs (in preparation), Harm (1963)

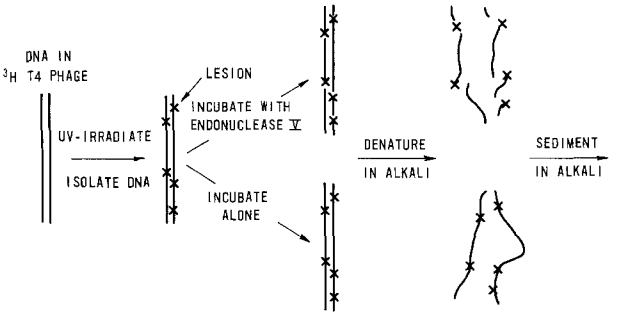

Fig. 1. Enzymatic assay for the detection of endonuclease V-sensitive sites. ^{14}C-labelled control DNA from non-irradiated T4, co-extracted with ^{3}H-labelled UV-irradiated T4, is not shown in this figure for simplicity. The ^{14}C-DNA is used as an internal control to detect non-specific strand breaks occurring during the endonuclease treatment, and as a molecular weight standard for the determination of the number of breaks introduced by the endonuclease treatment into the irradiated DNA

UV Irradiation. Phage particles suspended in phage buffer were irradiated with 254 nm UV light from a 15 W germicidal lamp (Childs and Birnboim, 1975). Near UV irradiation was carried out in a Schoeffel monochromator using a glass cuvette with a magnetic stirrer. To eliminate scattered far UV light a Mylar filter was used for irradiation at 313 nm and at 320 nm. The Mylar filter had an O.D. of 0.12 at 320 nm, 0.27 at 313 nm and 3.0 at 304 nm.

Preparation of Extracts of T4-Infected E. coli *with Endonuclease V Activity.* An overnight culture of *E. coli* H514 was diluted 100-fold in H-broth and grown for 2.5 h at 30° C with aeration, which yielded about 2×10^8 cells/ml. The bacteria were then infected with T4 at a multiplicity of infection of 3, and after incubation for a further 20 min at 30° C, NaCN (1 mM final concentration) was added and the culture was chilled in an ice bath. The cells were collected by low speed centrifugation, resuspended in DNA dialysis buffer, collected again by centrifugation and finally resuspended in one-tenth the original culture volume in DNA dialysis buffer. The cells were disrupted by sonication and the cell debris removed by centrifugation at 10,000 rpm for 30 min. The cell extracts (10 mg protein/ml) were stored at −17° C and retained sufficient activity for the assay described below for more than 12 months.

Phenol Extraction of DNA from T4 Phage. DNA was extracted at room temperature from T4 by mixing equal volumes of phage suspension and freshly distilled phenol saturated at 4° C with 0.5 M Tris pH 8.0. The aqueous phase was separated from the phenol phase by centrifugation at 10,000 rpm for 15 min and then dialysed against DNA dialysis buffer at 4° C for 16 h.

In vitro Enzymatic Assay to Detect UV Endonuclease-Sensitive Sites. The method described below is shown diagramatically in Figure 1 and is given in greater detail by Paterson (1977). The reaction mixture consisted of the following: 0.1 μg UV-irradiated ^{3}H-thymidine labelled T4 DNA ($2\text{–}5 \times 10^4$ dpm), 1.0 μg ^{14}C-thymidine labelled T4 control DNA ($1\text{–}2 \times 10^4$ dpm), 100 μg tRNA, and 20 μl of purified *Micrococcus luteus* UV endonuclease or 20 μl of *E. coli* H514 extract in a total volume of 140 μl. The mixture was incubated at 37° C for 30 min and then 100 μl of 1 M NaOH was added. [In experiments using the crude extract of *M. luteus* (equivalent to fraction 2 of Carrier and Setlow, 1970) the same procedure was adopted except that after incubation at 37° C for 30 min an additional 20 μl of extract was added and incubation was continued for a further 30 min before addition of NaOH.] The number of single strand breaks introduced into the UV-irradiated DNA by the preparations containing UV endonuclease activity was determined by a comparison of the molecular weight of the irradiated ^{3}H-labelled DNA with that of the control ^{14}C-labelled DNA after alkaline sucrose gradient sedimentation.

Photoreactivation. The same reaction mixture as described above was used except that 20 μl of photolyase, purified from *Streptomyces griseus* by A.P.M. Eker by the method of Eker and Fichtinger-Schepman (1975), was substituted for the UV endonuclease. The mixture was exposed to 300 to 420 nm light with a maximum intensity at 365 nm emitted from two GE black light tubes (F15T8/BLB) at a distance of 2.5 cm at 37° C for 30 min. A filter consisting of 5 mm of Lucite was placed between the sample and the black lights to minimize exposure of the sample to far UV radiation. No detectable single strand breaks or endonuclease V-sensitive sites were introduced by this photoreactivation treatment, since the molecular weight of the control ^{14}C labelled DNA was unaffected.

Results

UV Sensitivity of denV *Relative to* denV$^+$. In order to determine whether the UV sensitivity of *denV1* relative to *denV*$^+$ increases at wavelengths longer than 300 nm, a mixture of *denV1amB25* (gene 34) and *denV*$^+$*rb50* was irradiated at 254, 300, 313 and 320 nm. The use of the secondary mutations *amB25* and *rb50* permitted *denV1* and *denV*$^+$ to be irradiated together, thus ensuring equal UV exposure, but assayed separately on *E. coli* CR63(λh) and *E. coli* B respectively. The *amB25* and *rb50* mutations did not change the UV sensitivities of *denV1* and *denV*$^+$ respectively (data not shown). Two other UV sensitive mutants *denV110* and *uvsX102*, isolated in this laboratory (Childs, manuscript in preparation) were also tested for their sensitivity to 254 nm and 313 nm light, to determine whether increased relative sensitivity to near UV is characteristic of *denV* mutants. Both of these mutants were assayed on *E. coli* S/6.

The results (Table 2) confirm the finding of Winkler et al. (1962) and Cavilla and Johns (1964) that

Table 2. Relative sensitivity[a] of T4 UV sensitive mutants

Mutant	UV Wavelength			
	254 nm	300 nm	313 nm	320 nm
denV$^+$	1.0	1.0	1.0	1.0
denV1	2.3	3.1	3.8	3.4
denV110	2.3	NT[b]	4.1	NT[b]
uvsX102	1.8	NT[b]	1.7	NT[b]

[a] Relative sensitivity was calculated as the dose required for 99% inactivation of wt (*denV*$^+$) divided by the dose required for 99% inactivation of each mutant at each wavelength. Each figure is the mean of 2 or 3 determinations

[b] Not tested

the relative sensitivity of *denV1* compared to *denV*$^+$ is higher when irradiated with near UV light (300 nm) than it is when irradiated with far UV light (254 nm). The relative sensitivity reached a maximum of about 3.8-fold for 313 nm irradiation. The results also show that *denV1* and *denV110* have similar UV sensitivities to each other at 254 nm and 313 nm, and that the relative UV sensitivity of *uvsX102* to wild type is no higher at 313 nm than it is at 254 nm.

Comparison of UV Endonuclease Activity from T4 Infected Cells with UV Endonuclease from M. luteus. The in vitro enzymatic assay for endonuclease-sensitive sites in UV irradiated DNA normally uses purified UV endonuclease from *Micrococcus luteus* (Paterson et al., 1973; Paterson, 1975). We wished to determine whether endonuclease V of T4 from a crude extract of T4 infected *E. coli uvrA* could be used instead. To be useful such an extract should have little or no non-specific endonuclease activity in the assay and should introduce a similar number of breaks into 254 nm-irradiated DNA as purified UV endonuclease. *E. coli* H514 was chosen as the host as it lacks both endonuclease I activity, which would interfere with the in vitro enzymatic assay by increasing the noise-to-signal ratio, and the *E. coli* UV endonuclease.

DNA from bacteriophage T4 irradiated to a survival of about 30% at 320 nm and a survival of about 10% at 254 nm was treated with various *E. coli* and *M. luteus* preparations in the in vitro enzymatic assay. It was found that the extract of *E. coli* H514 infected with T4 *denV*$^+$ (wild type) introduced the same number of breaks into DNA from phage irradiated with 254 nm or 320 nm light as did the UV endonuclease purified from *M. luteus* (Table 3). The crude extract of *M. luteus* introduced almost as many breaks into the irradiated DNA as did the purified enzyme. The extracts of uninfected and *denV*-infected *E. coli* H514 had no detectable UV endonuclease activity. An indication of non-specific endonuclease activity can be obtained by comparing the molecular weights of the control DNA exposed to the different extracts (Table 3). It can be seen that the extract of uninfected *E. coli* H514 has no non-specific endonuclease activity in this assay. The other preparations all show some non-specific activity, including the purified endonuclease, but it is very small compared to their UV endonuclease activity. Therefore the extract of *denV*$^+$-infected *E. coli* H514 was used as a source of UV endonuclease in further experiments.

An incidental result of the experiments reported in this paper is that more single strand breaks (and/or alkali-labile bonds) are introduced into the DNA in proportion to the number of endonuclease V-sensitive sites by 320 nm irradiation than by 254 nm irradiation. An indication of this can be seen in Table 3 (see No extract treatment) and this was confirmed in subsequent experiments with higher UV doses; the average number of single strand breaks or alkali-labile bonds in three experiments was 13.5 ± 2.8 for every 100 endonuclease V-sensitive sites formed during 320 nm irradiation. There were no detectable single strand breaks formed during 254 nm irradiation.

Table 3. Comparison of different bacterial preparations for their UV endonuclease activity

Wavelength	Preparation	Weight average molecular weight of DNA $\times 10^{-6}$ daltons		Relative no. of breaks[a] introduced into irradiated DNA
		Control	Irradiated	
254 nm	*M. luteus* purified UV endonuclease	24.0	4.6	1.00
	M. luteus crude extract	22.7	6.0	0.71
	E. coli H514 T4 *denV*$^+$-infected	21.2	4.2	1.11
	E. coli H514 T4 *denV*-infected	22.8	20.0	0.03
	E. coli H514 uninfected	26.2	24.0	0.02
	No extract	26.7	24.4	0.02
320 nm	*M. luteus* purified endonuclease	22.8	10.3	1.00
	M. luteus crude extract	22.0	11.1	0.83
	E. coli H514, T4 *denV*$^+$-infected	24.0	10.3	1.03
	E. coli H514, T4 *denV*-infected	24.7	21.2	0.12
	E. coli H514 uninfected	27.6	23.2	0.13
	No extract	26.6	23.3	0.11

[a] The number of breaks introduced into UV irradiated DNA by each extract was calculated as described previously (Paterson, 1977) and is given relative to the number introduced by the purified *M. luteus* extract at each wavelength

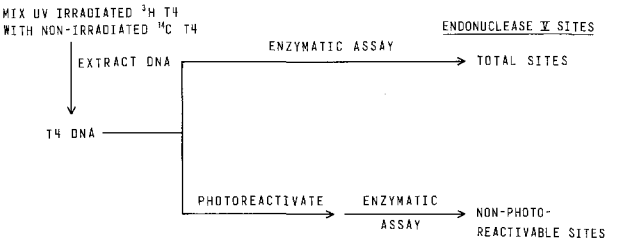

Fig. 2. Method for the detection of non-photoreactivable endonuclease V-sensitive sites in UV-irradiated T4

Non-Photoreactivable Endonuclease V-Sensitive Sites. The previous study by Cavilla and Johns (1964) suggested that during long wavelength UV irradiation of T4 a photoproduct is formed which can be recognized by endonuclease V but cannot be photoreactivated. We therefore designed an experiment to detect non-photoreactivable endonuclease V-sensitive sites in the DNA of UV irradiated T4 phage. An outline of the experiment is shown in Figure 2. T4 phage labelled with ^{3}H-thymidine was irradiated with either 254 nm or with 320 nm light and then mixed with non-irradiated ^{14}C-thymidine labelled phage. The DNA was extracted with phenol and then divided into two portions. One portion was assayed for total endonuclease V-sensitive sites. The second portion was first exposed to photolyase and light to reverse pyrimidine dimers in situ and then assayed for residual endonuclease V-sensitive sites. Photoreactivation should remove all of the pyrimidine dimers but in practice up to 10% may remain in the DNA (Cook and Worthy, 1972).

It was found that for wild type phage irradiated with 254 nm light, only 10% of the endonuclease V-sensitive sites could not be photoreactivated. However in the DNA from wild type phage irradiated with 320 nm light 47% of the endonuclease V-sensitive sites could not be photoreactivated (Table 4). This confirms that an unusual photoproduct is formed in the DNA of T4 irradiated at 320 nm, which is not detected in DNA from phage irradiated with 254 nm light.

T4 mutants With Different Base Compositions. Bacteriophage T4 is unusual in that it has glucosylated 5-hydroxymethylcytosine (5HMC) in its DNA instead of cytosine (Wyatt and Cohen, 1953; Volkin, 1954). Mutants of T4 exist which either have non-glucosylated 5HMC (*αgt βgt*, Georgopoulos, 1967) or cytosine instead of glucosylated 5HMC in their DNA (*alc*, Snyder et al., 1976). As cytosine gives rise to important UV induced photoproducts we repeated the previous experiment using these mutants.

It was found that virtually all the endonuclease-sensitive sites induced in both mutants by either 254 nm or 320 nm irradiation were photoreactivable (Table 4). Thus glucosyl-5HMC appears to be involved in the formation of the non-photoreactivable photoproduct.

UV Sensitivities of T4 Mutants Containing Cytosine or Non-Glucosylated 5HMC. In order to determine whether differences in base composition affect the UV sensitivity of T4, *denV* and *denV*$^{+}$ containing glucosylated 5HMC, non-glucosylated 5HMC or cytosine were irradiated at 254 and 313 nm. We found that differences in base composition did affect both the absolute UV sensitivities of *denV* and *denV*$^{+}$ and the relative sensitivity of *denV* compared to *denV*$^{+}$, but the differences were small (Fig. 3 and Table 5). The strains containing non-glucosylated 5HMC were about 20% more resistant to 254 nm and 313 nm light than the corresponding strains containing glucosylated 5HMC. The slight resistance of *denV*$^{+}$ containing non-glucosylated 5HMC compared to normal *denV*$^{+}$ at 254 nm was unexpected as in a previous study (Fukasawa, 1964) they appeared to have identical sensitivities. The cytosine containing strains were 17–36% more sensitive than the corresponding normal strains except for *denV*$^{+}$ containing cytosine which had the same sensitivity as normal *denV*$^{+}$ at 313 nm. The 17% increase in sensitivity to 254 nm irradiation of cytosine-containing *denV*$^{+}$ phage compared to normal *denV*$^{+}$ phage is similar to that obtained by E. Kutter (personal communication).

Table 4. Non-photoreactivable endonuclease V-sensitive sites in the DNA of UV-irradiated T4 phage. Each value is the mean of two determinations (±standard error) except for that of the cytosine containing phage

T4 Strain	% Sites non-photoreactivable	
	254 nm	320 nm
wild type	10.0±4.0	47.3±5.8
non-glucosylated (*αgt βgt*)	2.6±0.04	10.8±0.6
cytosine containing (*alc*)	2.6	6.6

Table 5. Effect of base composition on UV sensitivity of T4

Base	254 nm			313 nm		
	Sensitivity[a] of *denV*$^{+}$	Sensitivity[a] of *denV*	Rel. sensitivity *V*: *V*$^{+}$[b]	Sensitivity[a] of *denV*$^{+}$	Sensitivity[a] of *denV*	Rel. sensitivity *V*: *V*$^{+}$[b]
G5HMC	1.00	1.00	2.17	1.00	1.00	3.51
5HMC	0.78	0.85	2.36	0.86	0.78	3.25
cytosine	1.17	1.36	2.54	1.00	1.21	4.25

[a] Sensitivity of T4 containing non-glucosylated 5HMC or cytosine relative to glucosyl-5HMC-containing T4. For method of calculation see Table 2

[b] Dose required for 99% inactivation of *denV*$^{+}$ divided by dose required for 99% inactivation of *denV*

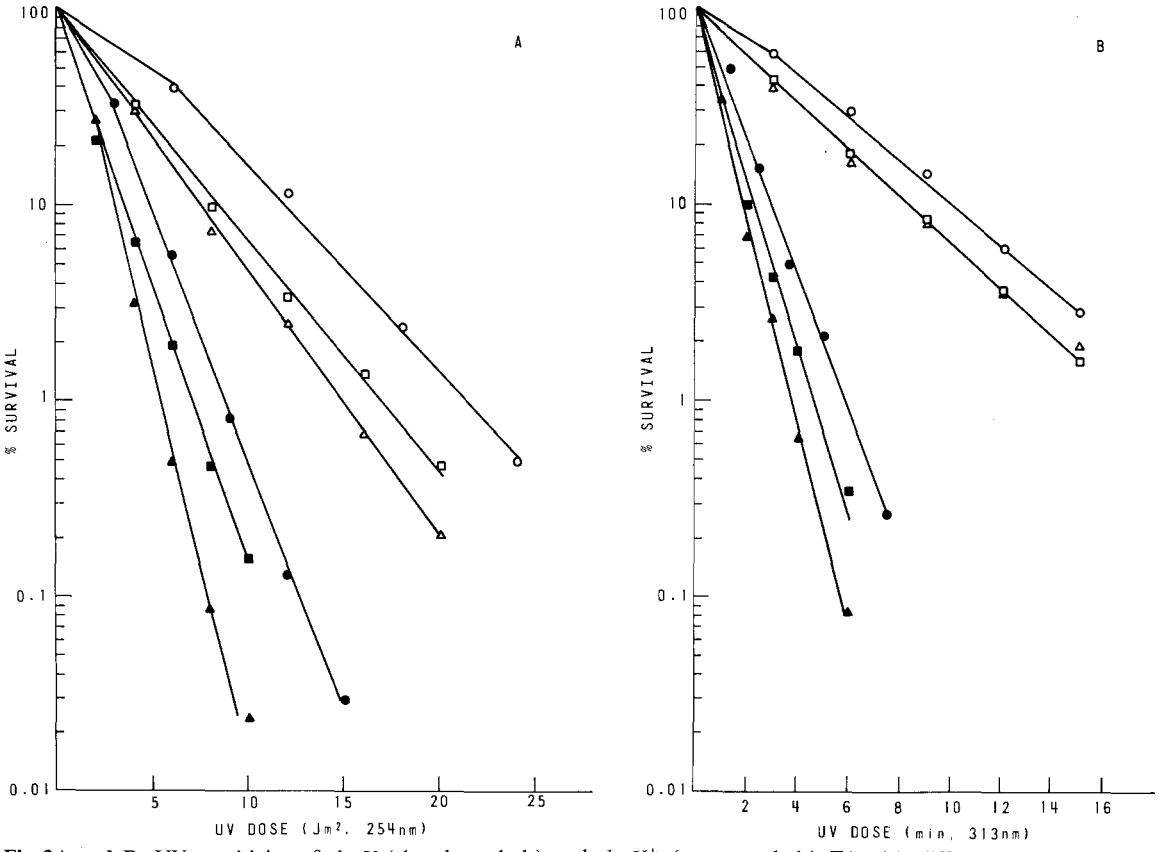

Fig. 3A and B. UV sensitivity of *denV* (closed symbols) and *denV*$^+$ (open symbols) T4 with different base compositions **A** 254 nm irradiation **B** 313 nm irradiation. ○ ● non-glucosylated 5HMC-containing phage, □ ■ glucosylated 5HMC-containing (normal) phage, △ ▲ cytosine-containing phage. One minute irradiation at 313 nm is equivalent to 5.2×10^3 J/m^2

Discussion

We have found that when wild type T4 is irradiated with 320 nm light, but not with 254 nm light, a photoproduct is induced in its DNA that can be recognized by endonuclease V but cannot be photoreactivated in vitro. Approximately half of the endonuclease V-sensitive sites induced by 320nm light were of this type (Table 4). We did not detect this photoproduct in T4 containing cytosine or non-glucosylated 5HMC irradiated with either 254 nm or 320 nm light. As glucosylation involves only 5HMC this implies that the photoproduct contains a glucosyl 5HMC residue. An obvious possibility is that the photoproduct involves a cross-link between glucose and either protein or DNA. However, if this is a lethal photoproduct and is only formed during irradiation with near UV, as our results and those of others (Winkler et al., 1962; Cavilla and Johns, 1964) suggest, then non-glucosylated phage should be considerably more resistant to 313 nm light than glucosylated phage, but should have the same sensitivity to 254 nm light as glucosylated phage. We found that non-glucosylated phage *were* more resistant to UV but the difference was small (~20%) and was observed at both 254 nm and 313 nm (Fig. 3 and Table 5). Thus it seems unlikely that glucose is directly involved in the formation of the photoproduct. A more likely explanation is that the photoproduct can be formed from either 5HMC or cytosine and that glucosylation prevents photoreactivation possibly by interfering with the binding of photolyase with the photoproduct. This would explain our failure to detect the photoproduct in non-glucosylated or cytosine-containing DNA and the similarity of the UV survival curves of the different strains. The photoproduct could be a dimer involving only 5HMC (or cytosine) or both 5HMC (or cytosine) and thymine. If it is a dimer containing either cytosine or 5HMC then it is not surprising that it can be recognized by endonuclease V, as this enzyme appears to be highly specific for pyrimidine dimers (Friedberg, 1972). The increased UV sensitivity of cytosine-containing phage compared to normal phage may be the result of an increased yield of cytosine photoproducts relative to 5HMC photoproducts (Setlow, 1970).

The relative sensitivity of *denV* compared to *denV*$^+$ at 254 nm is much lower than that found for

corresponding mutants of *E. coli; uvrA* mutants of *E. coli,* which lack UV endonuclease, are 10–30 fold more sensitive than wild type *E. coli* to 254 nm irradiation (Howard-Flanders et al., 1962; Hill, 1965). This may reflect a less efficient pathway of excision repair of UV-induced photoproducts from T4 DNA than from *E. coli* DNA. Similarly the increase in relative sensitivity of *denV* to near UV irradiation compared to far UV irradiation may indicate that the T4 excision repair pathway, involving endonuclease V, is able to remove near UV photoproducts more efficiently than it can remove far UV photoproducts. The relative sensitivity of a *uvsX* mutant, *uvsX102,* to wild type was the same at 254 nm and 313 nm (Table 2). This indicates that the pathway of repair involving the *uvsX* gene can also repair both near and far UV photoproducts but with similar efficiency.

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