Effect of UV Irradiation on Macromolecular Synthesis and Colony Formation in *Bacteroides fragilis*

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Irradiation of *Bacteroides fragilis* cells with far-UV light resulted in the immediate, rapid and extensive degradation of DNA which continued for 40 to 60 min after irradiation. During the degradation phase, DNA synthesis was decreased but was never totally inhibited. DNA degradation after irradiation was inhibited by chloramphenicol and caffeine. DNA synthesis in irradiated cells was reduced by chloramphenicol but resumed after 100 min at the same exponential rate as in irradiated cells without chloramphenicol. Irradiated cells continued to synthesize DNA for 40 min in the presence of caffeine but after this time DNA synthesis was completely inhibited and never recovered. RNA and protein synthesis were decreased by UV irradiation and the degree of inhibition was proportional to the UV dose. Colony formation was not affected immediately by UV irradiation and continued for a dose-dependent period before inhibition. There was an inverse relationship between UV dose and inhibition of colony formation which occurred sooner in cells irradiated with lower doses of UV light. The characteristics of DNA synthesis in *B. fragilis* cells after UV irradiation differ from those in wild-type *Escherichia coli* cells, where DNA synthesis is stopped immediately by UV irradiation, but resemble those in *E. coli recA* mutant cells where extensive degradation occurs following UV irradiation.

INTRODUCTION

DNA synthesis in *Escherichia coli* and a number of other aerobic bacteria is inhibited by far-UV radiation (254 nm) (Kelner, 1953; Swenson & Setlow, 1966; Swenson, 1976; Trgovcevic *et al.*, 1980). Inhibition of DNA synthesis occurs almost immediately. After a dose-dependent lag it recovers and proceeds at the same rate as in unirradiated cells. Photoreactivation largely relieves this inhibition, indicating that the inhibition is caused primarily by pyrimidine dimers (Hall & Mount, 1981). *RecA* mutants of *E. coli* extensively degrade their DNA after UV irradiation (Clark, 1973). Most of this degradation is due to the *recBC* DNAase (Willets & Clark, 1969; Satta *et al.*, 1979), and Williams *et al.* (1981) showed that purified *recA*⁺ protein inhibited the endonuclease and exonuclease activities of *recBC* DNAase on single-stranded DNA. DNA synthesis after UV irradiation was studied by Trgovcevic *et al.* (1980) in wild-type, *uvrA*, *recB*, *recA-recB* and *recA* *E. coli* strains and it was shown that the inhibition of DNA synthesis after UV irradiation depended on the *recA* gene being functional. In *E. coli* the UV-inducible *recA* protein has a molecular weight of 37800 (Horii *et al.*, 1980; Sancar *et al.*, 1980).

Irradiation of *Bacteroides fragilis* cells under anaerobic conditions results in the induction of a new 95000 molecular weight protein and the increased synthesis of two proteins with molecular weights of 70000 and 90000 (Schumann *et al.*, 1982). The induction of a 37000 to 40000 molecular weight protein by UV irradiation was not detected in *B. fragilis*. As the far-UV-induced systems in *B. fragilis* (Jones *et al.*, 1980; Jones & Woods, 1981; Slade *et al.*, 1981; Schumann *et al.*, 1982) seem to differ in certain respects from those in *E. coli*, we investigated the effect of UV irradiation on macromolecular synthesis and colony formation under anaerobic conditions. This appears to be the first report of the effect in an obligate anaerobe.

METHODS

Bacteria and media. These studies were carried out on a *B. fragilis* strain (Bf-2) which has been used in previous studies on far-UV irradiation (Jones *et al.*, 1980; Jones & Woods, 1981; Slade *et al.*, 1981, 1983; Schumann *et al.*, 1982). Brain heart infusion broth and agar, supplemented with haemin, menadione and cysteine (Holdeman & Moore, 1972) were used for bacterial propagation at 37 °C. Pre-reduced one-quarter-strength Ringer solution was used as a dilution buffer (Jones & Woods, 1981). Irradiation and radioactive labelling of the cells were done in a defined minimal medium (Varel & Bryant, 1974). The doubling time during exponential growth of *B. fragilis* in this minimal medium was 62 min at 37 °C. All manipulations were carried out under stringent anaerobic conditions in an anaerobic glove cabinet (Forma Scientific, Marietta, Ohio, USA).

UV irradiation. Overnight cultures of *B. fragilis* cells in brain heart infusion broth were diluted 100-fold in minimal medium and reincubated until the cultures reached a turbidity of 0.2 at 600 nm (1×10^8 to 2×10^8 c.f.u. ml⁻¹). Samples (9 ml) of the cultures were irradiated in open glass Petri dishes with a Fluotest Piccolo Hanau Quartz germicidal lamp which emitted the majority of its output at 254 nm. The dose-rate was measured with a Blak-Ray UV meter (model J-225; UV Products Inc., San Gabriel, Calif., USA) and samples were irradiated at a flux of $1.0 \text{ J m}^{-2} \text{ s}^{-1}$. Survival curves of bacteria irradiated with increasing doses were determined, and labelling experiments were carried out with cultures which were irradiated to different survival levels.

DNA, RNA and protein synthesis. DNA synthesis was determined by the incorporation of [2-¹⁴C]thymidine (4 µCi ml⁻¹; 1 µCi = 37 kBq) or [methyl-³H]thymidine (10 µCi ml⁻¹), RNA synthesis by the incorporation of [5,6-³H]uracil (15 µCi ml⁻¹), and protein synthesis by the incorporation of L-[³⁵S]methionine (20 µCi ml⁻¹) into cold TCA precipitable cellular material. The labelled chemicals were supplied by Amersham. The final concentrations of thymidine, uracil and methionine were 7, 15 and 25 µg ml⁻¹ respectively, which were saturating for both uptake and incorporation over 240 min.

The effect of UV irradiation on DNA, RNA and protein synthesis was determined in prelabelled and unlabelled cultures (Smith & O'Leary, 1968; Setlow & Setlow, 1970). In order to prelabel cells, overnight brain heart infusion broth cultures were inoculated into minimal medium and incubated until the cultures reached a turbidity of 0.15 at 600 nm. The label was added and the cultures were reincubated, until a turbidity of 0.2 at 600 nm was reached, before irradiation with UV light (prelabelled cells). In experiments involving unlabelled cells, the label was added to the culture immediately after irradiation. For dual labelling experiments, the cells were prelabelled for 60 min with [¹⁴C]thymidine. The labelled cells were then harvested by centrifugation, resuspended in fresh minimal medium without label and incubated for a further 50 min before irradiation and the addition of [³H]thymidine.

Effect of chloramphenicol and caffeine on DNA synthesis. The effect of chloramphenicol (5 µg ml⁻¹) and caffeine (1 mg ml⁻¹) on DNA synthesis after UV irradiation was determined with prelabelled and unlabelled cells. The inhibitors were not present during irradiation, but were added to minimal medium cultures immediately after irradiation. The minimal inhibitory concentrations of chloramphenicol and caffeine were 1 µg ml⁻¹ and 2.5 mg ml⁻¹, respectively.

Effect of UV irradiation on colony formation. Exponential phase cells in minimal medium were irradiated with UV doses of 0, 30, 40, 50 or 70 J m⁻² and reincubated at 37 °C under anaerobic conditions. Colony formation was determined after different time intervals by plating on brain heart infusion agar.

RESULTS

Effect of UV irradiation on DNA synthesis

UV irradiation of *B. fragilis* cells prelabelled with [¹⁴C]thymidine resulted in an initial rapid net loss of label from the TCA precipitable fraction (Fig. 1). The DNA degradation phase was followed after approximately 60 min by the net synthesis of DNA. Dual labelling experiments were carried out to determine whether DNA synthesis was masked during the DNA degradation phase (Fig. 2). DNA synthesis was decreased after UV irradiation, but never totally inhibited. This DNA synthesis was masked in the prelabelling experiments by the phase of extensive DNA degradation immediately after exposure to UV irradiation. Degradation of prelabelled DNA continued for 40 min post-irradiation before reaching a plateau between 40 and 160 min, when there was no net loss of [¹⁴C]thymidine from the DNA.

DNA synthesis was also determined in unlabelled cultures which were irradiated with increasing doses of UV light before the addition of [¹⁴C]thymidine. In these experiments DNA synthesis was measured by the net accumulation of label into TCA precipitable material. In unirradiated unlabelled *B. fragilis* cells exponential incorporation of [¹⁴C]thymidine similar to that in unirradiated prelabelled cells was observed 30 min after the addition of the label, which

Fig. 1

Fig. 2

Fig. 1. Effect of UV irradiation on DNA synthesis in prelabelled *B. fragilis* cells. The [^{14}C]thymidine was added to the cells 60 min before irradiation. The cells were irradiated and reincubated in the presence of the label. Unirradiated control (●); cells irradiated with UV doses of 50 J m^{-2} (11% survival) (○) and 70 J m^{-2} (1.2% survival) (Δ).

Fig. 2. Effect of UV irradiation on DNA synthesis in dual-labelled *B. fragilis* cells. Cells prelabelled with [^{14}C]thymidine for 60 min were harvested by centrifugation, resuspended in fresh medium without label and incubated for a further 50 min before UV irradiation (50 J m^{-2} , 11% survival) and the addition of [^3H]thymidine at time 0. Unirradiated prelabelled control reincubated in [^{14}C]thymidine at time 0 min (●). Irradiated cells with (Δ) and without (○) [^3H]thymidine added at the time of irradiation (time 0 min). Unirradiated cells with [^3H]thymidine added at time 0 (▲).

indicated that pool equilibrium of the thymidine precursor in the prototrophic *B. fragilis* cells was attained within that 30 min period. DNA synthesis was reduced after UV irradiation and showed linear kinetics for a dose-dependent period (Fig. 3). At higher UV doses there was a longer period of linear DNA synthesis. DNA synthesis during this linear phase also decreased with increasing UV doses. After irradiation to different survival levels, DNA synthesis resumed in all the cultures at the same exponential rate with the exception of the 1.2% survival culture. The time that elapsed before the resumption of exponential DNA synthesis was dose-dependent.

Effect of chloramphenicol and caffeine on DNA synthesis after UV irradiation

Experiments with prelabelled cells indicated that extensive degradation of DNA was inhibited by the addition of chloramphenicol immediately after UV irradiation (Fig. 4). In these prelabelled cells net DNA synthesis was detected 100 min after UV irradiation. Experiments with unlabelled cells showed that DNA synthesis after UV irradiation was markedly reduced by chloramphenicol but not inhibited completely. After 100 min DNA synthesis in chloramphenicol-treated irradiated cells resumed at the same exponential rate as that in irradiated cells without chloramphenicol. DNA synthesis in unirradiated cells was decreased by chloramphenicol and inhibited after 60 min. Chloramphenicol ($5 \mu\text{g ml}^{-1}$) inhibited protein synthesis immediately in unirradiated cells.

Caffeine treatment of prelabelled irradiated cells inhibited extensive DNA degradation immediately and DNA synthesis 40 min after UV irradiation (Fig. 5). DNA synthesis in unlabelled UV-irradiated cells was completely inhibited by caffeine 40 min after irradiation. Caffeine did not affect DNA synthesis in unirradiated cells.

Fig. 3. Effect of increasing doses of UV irradiation on DNA synthesis in unlabelled *B. fragilis* cells. Exponential phase cells were irradiated and labelled with [^{14}C]thymidine. Unirradiated control (●). Cells irradiated with UV doses of (J m^{-2}): 20 (86% survival) (○); 30 (62% survival) (△); 40 (26% survival) (▲); 50 (11% survival) (□) and 70 (1.2% survival) (■).

Fig. 4

Fig. 5

Fig. 4. Effect of chloramphenicol on DNA synthesis after UV irradiation. Cells prelabelled with [^{14}C]thymidine before UV irradiation with (△) and without (▲) chloramphenicol. Cells irradiated and then labelled with [^{14}C]thymidine with (□) and without (■) chloramphenicol. Unirradiated cells prelabelled with [^{14}C]thymidine with (○) and without (●) chloramphenicol. Cells were irradiated with a UV dose of 50 J m^{-2} (11% survival).

Fig. 5. Effect of caffeine on DNA synthesis after UV irradiation. Cells prelabelled with [^{14}C]thymidine before UV irradiation with (△) and without (▲) caffeine. Cells irradiated and then labelled with [^{14}C]thymidine with (□) and without (■) caffeine. Unirradiated cells prelabelled with [^{14}C]thymidine with (○) and without (●) caffeine. Cells were irradiated with a UV dose of 50 J m^{-2} (11% survival).

Fig. 6. Effect of UV irradiation on colony formation by *B. fragilis* cells under anaerobic conditions. Cells were irradiated with increasing doses of UV and samples were plated onto brain heart infusion agar at various times after irradiation. UV doses were (J m^{-2}): 30 (62% survival) (○); 40 (24% survival) (□); 50 (12% survival) (■) and 70 (1.5% survival) (△). Unirradiated control (●).

Effect of UV irradiation on RNA and protein synthesis

UV irradiation of *B. fragilis* cells resulted in a dose-dependent decrease in RNA and protein synthesis. Protein synthesis was less sensitive than RNA synthesis to UV irradiation. After irradiation at 50 J m^{-2} (11% survival) RNA synthesis was reduced by 42% compared with the unirradiated control and increased linearly for approximately 60 min before it resumed exponentially at a somewhat lower rate than in the unirradiated control. At the same dose protein synthesis was only reduced by 28% and continued in an exponential fashion. At 1.2% survival both RNA and protein synthesis were markedly reduced and showed linear kinetics until the end of the experiment (160 min).

Effect of UV irradiation on colony formation

The ability of *B. fragilis* to form colonies on agar medium was not affected immediately by UV irradiation (Fig. 6). The c.f.u. continued to increase in number for a dose-dependent period before colony formation was inhibited. Inhibition of colony formation occurred sooner in cells irradiated with lower doses of UV light than in cells irradiated with high doses of UV light.

DISCUSSION

Experiments with the anaerobe *B. fragilis* on the effect of UV irradiation on DNA synthesis support the conclusion of Setlow & Setlow (1970) that there is no unique best method for investigating macromolecular synthesis in irradiated bacteria. Rapid degradation of DNA immediately after UV irradiation of *B. fragilis* cells was only detected with prelabelled cells. This extensive degradation masked the discovery which was made with unlabelled cells that DNA synthesis was decreased, but never totally inhibited, by UV irradiation and continued during the degradation phase.

The characteristics of DNA synthesis in *B. fragilis* cells irradiated with far-UV light under anaerobic conditions differ from those reported for *E. coli*, where DNA synthesis is stopped completely and immediately by UV irradiation (Kelner, 1953; Swenson, 1976; Hall & Mount, 1981). This inhibition of DNA synthesis in *E. coli* cells is dependent on a functional *recA* gene product (Trgovcevic *et al.*, 1980). We previously reported that UV irradiation of *B. fragilis* cells

did not result in the induction of a 37000 to 40000 molecular weight protein analogous to the *recA* protein in *E. coli* (Schumann *et al.*, 1982). The discovery that UV irradiation under anaerobic conditions did not inhibit DNA synthesis supports our previous conclusion that *B. fragilis* cells do not have an *E. coli recA*-type system.

Although some DNA degradation does occur in wild-type *E. coli* cells after UV irradiation it is limited (Swenson, 1976). Extensive 'reckless' DNA degradation occurs in *recA* mutants following UV irradiation (Clark, 1973). A similar extensive degradation of DNA after UV irradiation was observed in wild-type *B. fragilis* cells. In *E. coli recA* mutants this DNA degradation reflects the action of an uncontrolled *recBC* DNAase (Williams *et al.*, 1981). *RecA* protein and *recBC* DNAase are not the only two enzymes involved in DNA degradation in *E. coli*. Excessive degradation following UV- or X-irradiation has been reported in *ras*, *polA* and *uvrD* mutants (Ogawa *et al.*, 1968; Boyle & Setlow, 1970; Youngs & Bernstein, 1973). In these strains, 75 to 90% of the degradation is accounted for by *recBC* DNAase, with the remainder attributed to other nucleases (Youngs & Bernstein, 1973). DNA degradation in *E. coli recA* mutants is greatly reduced by caffeine (Shimada & Takagi, 1967). Caffeine also inhibited DNA degradation in *B. fragilis*.

The inhibition by chloramphenicol of the extensive DNA degradation that occurs after UV irradiation in *B. fragilis* suggests that protein synthesis is required for degradation. In *E. coli* excision occurs in the presence of chloramphenicol (Swenson & Setlow, 1966). Chloramphenicol reduced DNA synthesis in irradiated *B. fragilis* cells but did not inhibit it completely. Although chloramphenicol prevents the resumption of DNA synthesis in irradiated *E. coli* (Swenson, 1976), Swenson & Setlow (1966) reported that in a radiation-resistant *E. coli* B strain DNA synthesis is resumed at the same time in irradiated cells with and without chloramphenicol. In *Haemophilus influenzae* X-rays induced degradation of DNA but the DNA breakdown was not inhibited by chloramphenicol (Stuy, 1961). DNA synthesis in irradiated *B. fragilis* cells treated immediately with chloramphenicol is analogous to stable DNA replication in *E. coli* in that it is an abnormal, UV-induced DNA synthesis which can occur in the absence of protein synthesis (Kogoma & Lark, 1970, 1975; Lark & Lark, 1978). However, *B. fragilis* differs from *E. coli* in that in *E. coli* protein synthesis is necessary during a 40 min period after irradiation before chloramphenicol addition for the initiation of stable DNA synthesis (Kogoma *et al.*, 1979).

In *B. fragilis* colony formation was not inhibited immediately by UV irradiation and there was an inverse relationship between UV dose and inhibition of viability. An initial transient increase in colony formation after UV irradiation has also been reported in *E. coli* under conditions where DNA synthesis was inhibited in virtually all the cells (Okagaki, 1960; Smith, 1969). Smith (1969) observed that with both UV-sensitive and UV-resistant *E. coli* mutants there was an inverse relationship between UV dose and inhibition of colony formation.

These basic studies on *B. fragilis* provide a starting point for future work on UV repair and recombination in this important anaerobe. At present investigations are hampered by our inability to isolate UV-sensitive or UV-resistant mutants in spite of an extensive screening programme. Our results on the characteristics of DNA synthesis after UV irradiation and the difficulty experienced in isolating mutants in general in *B. fragilis* suggest that the UV repair and recombination systems in this anaerobe may differ from those in *E. coli*, which tend to be accepted as typical for bacteria in general.

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