Pyrimidine Dimer Excision and DNA Degradation during Liquid Holding Recovery in Ultraviolet-irradiated *Escherichia coli* K12 *uvr⁺rec*

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The survival of ultraviolet-irradiated *Escherichia coli* K12 *uvr⁺rec* was increased by post-irradiation incubation in phosphate buffer. During this incubation both dimer excision and DNA breakdown were inhibited. It is suggested that the bacteria coped with the remaining dimers in a manner which did not involve excision.

INTRODUCTION

Certain strains of ultraviolet (u.v.)-irradiated *Escherichia coli* show increased survival when incubated after irradiation in a non-nutrient medium (Ganesan & Smith, 1968; Moss & Davies, 1974). Since this effect is pronounced in bacteria of the *uvr⁺rec* genotype, the phenomenon has been interpreted as being due to an increased excision of u.v.-induced pyrimidine dimers during prolonged incubation of the irradiated bacteria in a non-nutrient liquid before plating on to a nutrient medium (Ganesan & Smith, 1969; Harm, 1968). However, excision repair requires a carbon source (Setlow, 1967) and is profoundly inhibited during 1 h incubation without glucose (Setlow & Carrier, 1968).

In this study bacterial survival, dimer excision and DNA degradation in *Escherichia coli* K12 AB2487 *uvr⁺rec* during post-irradiation incubation of bacteria for 8 h in phosphate buffer were measured. Our results indicate that the number of bacteria surviving increased while both dimer excision and DNA degradation were inhibited.

METHODS

Bacterial strain and cultivation. *Escherichia coli* K12 strain AB2487 *uvr⁺rec leu arg pro his thr thy thi*, kindly supplied by A. K. Ganesan, Department of Radiology, Stanford University, U.S.A., was used. The liquid minimal growth medium was that described by Ganesan & Smith (1968). Complete medium was made by adding Difco vitamin-free Casamino acids (0.25 %, w/v) and thymine and thiamin (each at 2 µg ml⁻¹) to the minimal growth medium. This medium was solidified for plating with 1.5 % (w/v) agar. Liquid holding was done in a sodium-potassium phosphate buffer, pH 7.0, as described by Castellani, Jagger & Setlow (1964).

Bacteria were cultivated at 37 °C on a rotary shaker and, at the beginning of the exponential phase of growth (5×10^7 to 8×10^7 bacteria ml⁻¹), were harvested, washed and resuspended in minimal medium or buffer for irradiation.

Ultraviolet irradiation. Bacteria were u.v.-irradiated with a germicidal lamp (Philips TUV 15W), at an intensity of 0.63 J s⁻¹ m⁻². A dose-rate meter model IL 254 (International Light, Newburyport, Massachusetts, U.S.A.) was used. Bacterial suspensions were irradiated in 1 to 2 mm thick layers in Petri dishes with manual stirring.

Pyrimidine dimer estimation. Bacteria were pre-labelled with 0.5 µCi [2-¹⁴C]thymine ml⁻¹ (specific activity 44 µCi mmol⁻¹) for u.v.-photoproduct estimation. Pyrimidine dimers were estimated by two-dimensional paper radiochromatography as described by Carrier & Setlow (1971). The activity of paper strips was measured in dioxan scintillation mixture in a Packard Tricarb 2450 liquid scintillation spectrometer.

Fig. 1

Fig. 2

Fig. 1. Survival of *Escherichia coli* K12 AB2487 *uvr*⁺*rec*. Exponentially growing bacteria (5×10^7 ml⁻¹ = s_0) were irradiated at 10 J m^{-2} and then divided into two parts: one part was incubated in complete medium (●); the other in liquid holding medium (▲). The number of bacteria at time t (s) was estimated by colony forming ability.

Fig. 2. Excision of pyrimidine dimers in *Escherichia coli* K12 AB2487 *uvr*⁺*rec*. Bacteria were pre-labelled with [2-¹⁴C]thymine and in the early exponential phase of growth irradiated at 30 J m^{-2} . Post-irradiation incubation was in complete medium (●) or in liquid holding medium (▲).

Fig. 3

Fig. 3. DNA degradation in *Escherichia coli* K12 AB2487 *uvr*⁺*rec*. For conditions, see legend to Fig. 2. An unirradiated control (■) showed spontaneous degradation.

DNA degradation. DNA degradation was estimated by measuring the amount of radioisotope remaining in the acid-insoluble fraction from bacteria uniformly labelled with [2-¹⁴C]thymine by growth in complete medium supplemented with $0.2 \mu\text{Ci}$ precursor ml⁻¹. Samples (0.5 ml) were mixed with 0.5 ml 10% (w/v) trichloroacetic acid in an ice bath. After keeping at 0 °C for a few hours, the mixtures were filtered and the precipitates washed twice in cold 10% trichloroacetic acid and twice in water on SYNPOR 6 (0.40 μm) membrane filters. The radioactivity on the filters was measured in a toluene scintillation mixture.

RESULTS AND DISCUSSION

The survival of irradiated bacteria incubated in phosphate buffer was significantly higher than the survival of those incubated in complete medium (Fig. 1). On the other hand, enzymic processes involved in dimer excision and DNA degradation occurred more slowly in buffer (Figs 2 and 3). About 40% of the induced dimers were excised after incubation in buffer for 3 h, and thereafter no further excision occurred (Fig. 2). The rate of degradation of DNA in irradiated bacteria incubated in buffer was less than in complete medium, but it continued during the whole period of incubation (Fig. 3).

It is not clear how bacteria coped with residual dimers. It might be presumed that they excised dimers after being transferred to nutrient plates. However, the results obtained by Ganesan & Smith (1968) indicate that liquid holding recovery occurs in a non-nutrient medium since it is interrupted by the addition of nutrients. This, as well as fast restoration of DNA synthesis after transferring bacteria to nutrient medium (Swenson & Setlow, 1966), renders the above explanation unlikely. It is also necessary to take into consideration the fact that dimer excision is measured in bacteria which predominantly do not survive. Thus an objection may be that the data do not represent what happens in those bacteria which recover in liquid holding. However, similar levels of residual dimers detected either in replicating and non-replicating or in living and dead parts of populations do not support this objection (Meyn *et al.*, 1974; Schenley, Fisher & Swenson, 1976; Sedliaková *et al.*, 1977). We therefore suggest that the irradiated bacteria incubated in buffer coped with residual dimers without excising them. It appears that incubation conditions after u.v.-irradiation which cause the inhibition of DNA breakdown as well as dimer excision aid the recovery of *uvr⁺rec* *E. coli*.

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