SHORT COMMUNICATIONS

Normal Excision of Pyrimidine Dimers after Thymine Starvation in *Micrococcus radiodurans*

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(Received 7 February 1977)

INTRODUCTION

The extraordinary ultraviolet (u.v.) radiation resistance of *Micrococcus radiodurans* is due to the highly effective dark-repair of pyrimidine dimers and involves both an excision (Boling & Setlow, 1966) and a recombination repair mechanism (Moseley, Mattingly & Copland, 1972). This high resistance to u.v. radiation, which is about 20 times greater than in repair-competent *Escherichia coli* (Setlow & Duggan, 1964), makes *M. radiodurans* an especially attractive model for studying repair processes. The availability of thymine-dependent mutants of *E. coli* and *M. radiodurans* makes it possible to study the influence of thymine starvation on the course of post-irradiation repair processes. Incubation of *E. coli* B/r *thy* in a medium without thymine results in an exponential decrease in the number of viable bacteria (Barner & Cohen, 1954) and in changes in the sensitivity of bacteria to u.v.-irradiation (Smith, Hodgkins & O'Leary, 1966). More recently, it has been shown that pre-irradiation inhibition of DNA synthesis induced by treatment with cytidine (Brozmanová, Mašková & Sedliaková, 1973) or by starvation for thymine (Sedliaková, Mašek & Brozmanová, 1972) causes a reduction in the amount of dimer excision. The extent of this reduction depends on the duration of the pre-irradiation thymine starvation period (Balgavý, Mašek & Turek, 1975). However, by simultaneous starvation for thymine and amino acid before u.v.-irradiation, a reduction in dimer excision can be induced in various excision-proficient strains of *E. coli*. The starvation need cause neither death of the bacteria during the treatment nor an increase in their sensitivity to u.v.-irradiation (Sedliaková *et al.*, 1974).

The purpose of the present study was to find out whether dimer excision after thymine starvation is affected in a similar manner in *M. radiodurans*.

METHODS

Organism. For the thymine-dependent mutant *Micrococcus radiodurans* T₈⁻ we are indebted to Dr J. G. Little, York University, Downsview, Toronto, Canada.

Media. The synthetic (SM) medium was that of Little & Hanawalt (1973) supplemented with sodium citrate (11 µg ml⁻¹) and with thymine (2 µg ml⁻¹) when required (SM-T⁺). The pH was adjusted to 6.8. Agar plates were prepared by solidifying SM medium supplemented with thymine (20 µg ml⁻¹), with 1% (w/v) agar. For washing the bacteria and diluting suspensions, we used a 0.067 M-phosphate buffer, pH 7.0, described by Moseley *et al.* (1972). Bacteria were irradiated in phosphate buffer which contained (per litre distilled water): KH₂PO₄, 0.5 g; K₂HPO₄·3H₂O, 0.65 g.

Measurement of growth. Densities of bacterial suspensions were measured in a Specol spectrophotometer at 650 nm.

Preparation of bacterial suspensions. A 24 h culture was diluted with fresh medium to an extinction of 0.08 and incubated by shaking at 37 °C until the early-exponential phase of growth was reached (about 3 × 10⁷ bacteria ml⁻¹).

Fig. 1

Fig. 2

Fig. 1. Excision of pyrimidine dimers from *Micrococcus radiodurans* T₂⁻ after a u.v. dose of 300 J m⁻²: exponentially growing bacteria (●); bacteria pre-starved for thymine for 180 min (▲) or 240 min (■). The survival of pre-starved bacteria was 60% and 20% respectively.

Fig. 2. Degradation of DNA in unirradiated (open symbols) and irradiated (filled symbols) *Micrococcus radiodurans* T₂⁻: exponentially growing bacteria (○, ●); bacteria pre-starved for thymine for 180 min (△, ▲) or 240 min (□, ■). A u.v. dose of 300 J m⁻² was used in all experiments.

Irradiation of bacterial suspensions. Bacteria were irradiated with a dose of 300 J m⁻² at 254 nm from two Philips TUV 30 W germicide lamps. The incident dose rate was measured on a Latarjet N 90 dose-rate meter.

Determination of pyrimidine dimers. Bacteria that had been growing for 18 h in SM-T⁺ medium containing [2-¹⁴C]thymine (0.7 µCi ml⁻¹) and had reached the early-exponential phase of growth were resuspended in cold phosphate buffer and u.v.-irradiated. Immediately after irradiation concentrated SM-T⁺ medium was added to the bacterial suspension. After various periods of post-irradiation incubation, samples were taken into cold trichloroacetic acid [TCA, final concentration 5% (w/v)]. Pyrimidine dimers were determined as described by Sedliaková, Mašek & Bernátová (1971).

For experiments involving thymine starvation, part of the culture in the exponential phase of growth was filtered and resuspended in a medium lacking thymine. After 180 or 240 min, the bacteria were transferred into cold phosphate buffer, and then irradiated and treated in the same way as the non-starved control.

The radioactivity of the photoproducts was determined by measuring the activity of chromatogram strips in toluene scintillation liquid using a Packard Tricarb liquid scintillation spectrometer.

Degradation of DNA. Bacteria were grown overnight in SM-T⁺ medium supplemented with [2-¹⁴C]thymine (0.5 µCi ml⁻¹) to the same extinction as in experiments for the determination of photoproducts. They were then transferred into non-radioactive SM-T⁺ medium and incubated for 90 min at 37 °C in order to exhaust the radioactive cell pools. After filtration the bacteria were resuspended in an equal volume of cold phosphate buffer and u.v.-irradiated. Immediately after irradiation concentrated SM-T⁺ medium was added to the irradiated culture (and to an unirradiated one) and incubation was continued in the dark at 37 °C. Samples (0.1 ml) were taken at intervals on to Whatman no. 3 paper discs. The decrease in radioactivity in the trichloroacetic acid-insoluble material on the discs, measured in toluene scintillation liquid, represented the extent of DNA degradation in *M. radiodurans* T₂⁻. Degradation in pre-starved samples was determined in the same manner.

RESULTS AND DISCUSSION

Dimer excision was equally efficient in both exponentially growing bacteria and bacteria incubated without thymine before u.v.-irradiation (Fig. 1). However, only 20% of the bacteria pre-starved for thymine for 240 min survived the dose of 300 J m^{-2} whereas all the exponentially growing bacteria survived.

The decrease in survival observed in bacteria incubated without thymine before u.v.-irradiation was accompanied by an increased breakdown of DNA (Fig. 2). This may be due to a defect in either the repolymerization or the rejoining steps involved in the excision repair process. Alternatively, damage produced by thymineless pre-incubation itself might trigger the action of exonucleases. The latter supposition is supported by the finding that breakdown of DNA after thymineless incubation occurred in unirradiated cells (Fig. 2).

Unlike the situation in *E. coli*, pre-incubation without thymine did not influence dimer excision in *M. radiodurans*. Although the reason for this difference is not clear, some evidence has been provided by experiments in which *E. coli* was irradiated with two separate u.v. exposures, the first being preceded by starvation for thymine and amino acid and the second following 2 h incubation in complete medium. Bacteria were unable to perform efficient excision from the DNA synthesized before thymine and amino acid deprivation but could do so from the DNA synthesized after such treatment (Setlow & Carrier, personal communication). These results indicate that the effect of thymine starvation on dimer excision is not due to enzymic deficiency. Moreover, thymine starvation of *E. coli* before u.v.-irradiation reduces photoreactability of dimers (Ghosh & Bhattacharjee, 1975). This suggests that in *E. coli* pre-incubated without thymine, dimers lose substrate activity for both u.v.-endonuclease and the photoreactivating enzyme, whereas in *M. radiodurans* treated in the same way loss of substrate activity of dimers for u.v.-endonuclease does not occur.

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