PERSISTENCE OF THYMINE DIMERS IN THE REPLICATED DNA OF *ESCHERICHIA COLI* B/r Hcr$^+$

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1. Introduction

Pyrimidine dimers, the most serious u.v. lesions [1], can be either removed from DNA molecules by excision [2,3] or tolerated by postreplication repair processes [4]. In bacterial cells pyrimidine dimers are removed almost quantitatively [5]. Therefore it has logically been concluded that in these cells excision repair fulfills a decisive role in restoration processes.

Previously we have reported that the excision activity of *E. coli* B/r Hcr$^+$ may be depressed by pre-irradiation starvation for thymine [6]. More recently we have shown that a simultaneous starvation for thymine and amino acid (AA$^-$T$^-$) applied prior to u.v. irradiation leads to depression of thymine dimer excision in various excision-proficient strains of *Escherichia coli* when proper starvation conditions are employed [7]. We have also shown that the starvation need not cause either death of cells during the treatment or a decrease of surviving ability [8,7].

The above-mentioned findings were in contradiction with the suggested significance of excision repair for the restoration of bacterial cells. However, it might be objected that excision was measured in the whole population of which only a small fraction could survive. Though in exponentially growing cultures the whole population is active in excision, it might be possible that the applied pretreatment selectively inhibits some metabolic steps in a certain part of the population.

In order to exclude the possibility that the observed depression of excision reflected the activity of the prevailing part of population inactive in DNA replication while in the replicating fraction dimers were removed efficiently, the amounts of dimers in the DNA strands both active and inactive in replication were determined separately. In this report proof is presented that in both types of DNA strands similar amounts of dimers remain unexcised.

2. Materials and methods

2.1. *Bacterial strain and cultivation conditions*

Bacterial strain of *Escherichia coli* B/r thy$^-$ trp$^-$ Hcr$^+$ was used. Cells were density and radioactively labelled for several generations. Density labelling was achieved by growing the cells in a heavy medium which contained 0.1% $^{15}NH_4Cl$ (99% atom purity) instead of 0.2% $^{14}NH_4Cl$ and 0.1% [^{13}C]glucose (78% atom purity) instead of 1% [^{12}C]glucose. Radioisotopic labelling of the DNA was accomplished by growing the cells in the heavy medium supplemented with 20 μCi [6-^{3}H] thymine/ml.

Cells in the exponential phase of growth (1–3 × 10^8 cells/ml) were chased for 20 min by the addition of [^{12}C]glucose (2% final concentration) and then transferred to the light medium without essential components (thy$^-$ trp$^-$). In this medium the cells were incubated for 90 min and then irradiated with a dose of 300 ergs/mm^2 (survival in untreated culture was 4.5%; in the treated culture 2.7%). After exposure incubation was continued in a fully supplemented light medium (no radioisotopic label) in which 2 μg thymine/ml was substituted for by 4 μg thymidine/ml.

2.2. *Ultraviolet irradiation*

A Philips TUV 15W germicidal lamp emitting pre-

dominantly light of a wave length of 2537 Å served as a source of radiation. The incident dose rate was 6.3 ergs/sec. Depth of the irradiated layer was 1–2 mm; the suspension was manually stirred during the irradiation.

2.3. *Isopycnic CsCl centrifugation and thymine dimer estimation*

DNA was isolated by the lysozyme-pronase procedure and separated by centrifugation in an equilibrium CsCl gradient as described previously [9].

In separated fractions of DNA after dialysis thymine dimer content was determined by two-dimensional paper radiochromatography [10]. For this purpose the dialysed pooled fractions after its drying were hydrolysed with 0.2 ml 98% HCOOH at 175°C for 45 min. The radioactivity of 10 mm wide strips of Whatman No 1 paper was measured (Packard, TRICARB 3375) each in 5 ml dioxan scintillator with 0.3 ml of water.

3. Results and discussion

To distinguish the two types of DNA strands, cells were grown in ^{13}C, ^{15}N medium and both the unreplicated (HH) and replicated (HL) strands were separated by ultracentrifugation in CsCl gradients (fig.1).

In our previous experiments *E. coli* B/r Hcr$^+$ cells irradiated by a dose dimerizing 0.15% of thymine exhibited complete excision in the exponentially growing culture [11] and an almost complete depression of excision in the prestarved culture [8,7]. In the experiments described here depression of dimer excision was less pronounced, probably due to different conditions (growth in heavy medium with a low level of glucose, transfer to light medium). However, there was little difference in dimer contents between DNA active and inactive in replication. As it can be seen from fig.2, the amounts of dimers in both replicated (HL) and unreplicated (HH) DNAs were similar to one another.

In the Oak Ridge National Laboratory an ingenious attempt was performed which made it possible to determine the extent of removal of dimers from the DNA synthetized both before and after the treatment. Cells were prelabelled with [^{3}H] thymine, starved for 2 h for thymine and amino acid and u.v. irradiated;

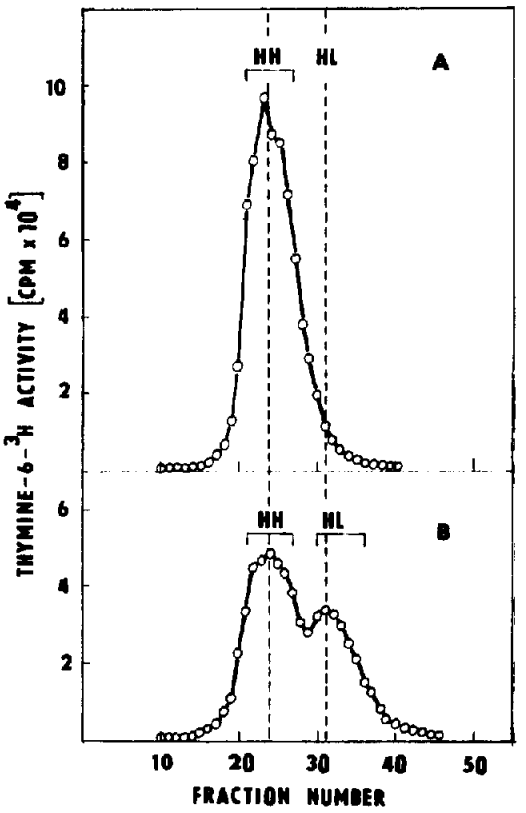

Fig.1. CsCl gradient profiles of DNA from AA$^-$T$^-$ pretreated and u.v. irradiated cells of *E. coli* B/r thy$^-$ trp$^-$ Hcr$^+$. Immediately after irradiation (A) and 3 h after irradiation (B) samples were withdrawn and DNA was isolated and separated by centrifugation in CsCl gradients. After fractionation the indicated top fractions of heavy-heavy (HH) double strands and hybrid (HL) double strands were pooled for the determination of thymine dimers.

then the cells were labelled for another 2 h with [^{14}C] thymine and once more u.v. irradiated. The culture of cells was found unable to perform efficient excision from the DNA synthesized *before* starvation (^{3}H-label), but was able to perform efficient excision from the DNA synthesized *after* the treatment (^{14}C-label) [12].

Data presented here are in good agreement with the above finding. They are also in conformity with the observation that almost similar amounts of dimers remain unexcised in both replicated and unreplicated DNA strands in u.v. irradiated Chinese hamster ovary

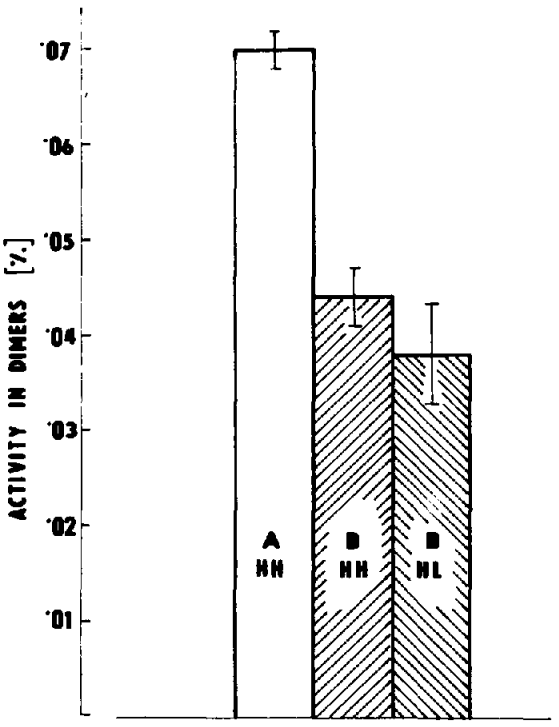

Fig.2. Thymine dimer content in DNA from AA^-T^- pretreated and u.v.-irradiated *E. coli* B/r thy^- trp^- Hcr^+ cells. Fractions indicated in fig.1 were pooled and dimers were determined: (A) immediately after u.v. in HH DNA; (B) 3 h after u.v. in both HH (unreplicated) and HL (replicated) DNA separately. The results are averages of four experiments.

cells [13] as well as with the finding that similar amounts of dimers may be detected in both surviving and dead fractions of the u.v. irradiated cells of *E. coli* B/r when separated by centrifugation in neutral sucrose gradient [14].

Excision-proficient bacterial cells similar to mammalian cells thus seem to successfully tolerate even great amounts of unexcised lesions.

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