THYMINE-DIMER EXCISION AFTER THE PREIRRADIATION INHIBITION OF DNA SYNTHESIS

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1. Introduction

Ultraviolet radiation impairs cells mainly by the production of pyrimidine dimers in DNA molecules. These lesions can either be removed by excision or tolerated by postreplication repair (for review, see [1]). Excision has been assumed to be a highly efficient tool enabling the cells to cope with a great number of UV lesions [2]. Thus it was surprising to find that the excision of dimers was rather depressed after amino acidless pretreatment which considerably enhanced the fraction of surviving cells [3].

In this paper the influence of the pre-irradiation inhibition of DNA synthesis on thymine-dimer excision is reported. As demonstrated, the excision process can be considerably inhibited by the inhibition of DNA synthesis before irradiation.

2. Materials and methods

2.1. *Bacterial strain and cultivation*

The cells of *Escherichia coli* B/r Hcr$^+$ thy$^-$ trp$^-$ were used. The medium and cultivation conditions were already described [4].

Logarithmically growing cells prelabelled with thymine-2-^{14}C were harvested by membrane filtration, washed, resuspended and pretreated as indicated below. The suspensions were irradiated by UV and postincubated for 120 min. Samples were withdrawn during this period for assay of thymine dimers.

2.2. *UV irradiation and determination of thymine dimers.*

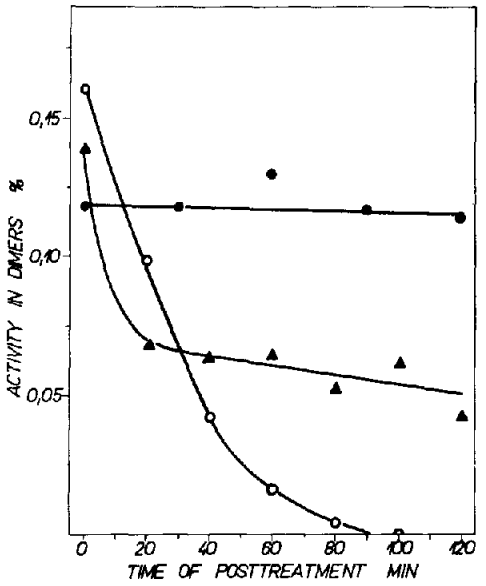

Fig. 1. Thymine-dimer excision after the preirradiation inhibition of DNA synthesis. Logarithmically growing cells were harvested by membrane filtration, resuspended and pretreated for 120 min either in the absence of thymine, or in the presence of 50 μg/ml of cytidine. Then they were irradiated by 750 ergs/mm^2 and cultivated for another 120 min in the complete medium without cytidine. During the posttreatment samples were withdrawn and the percentage of thymine dimerized was determined. (○–○–○) log growing cells; (●–●–●) T$^-$; (▲–▲–▲) Cyd$^+$.

Thymine dimers were determined radiochromatographically in the fraction precipitable in trichloroacetic acid. The conditions of UV irradiation and the radiochromatographic technique were described elsewhere [3].

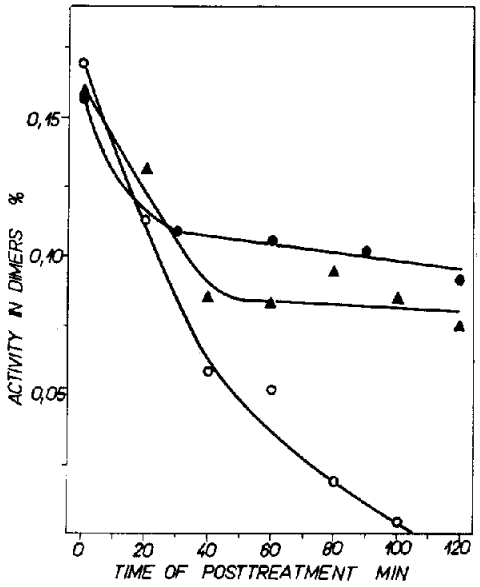

Fig. 2. Thymine-dimer excision after preirradiation inhibition of both the DNA and the protein syntheses. The cells were handled as above (see fig. 1) except that they were pretreated either in the amino acidless-thymineless ($AA^- T^-$) medium or in the amino acidless medium supplemented with 50 μg/ml of cytidine ($AA^- Cyd^+$). (o—o—o) AA^-, (•—•—•) AA^-T^-, (▲—▲—▲) $AA^- Cyd^+$.

3. Results and discussion

When the logarithmically growing cells of *E. coli* B/r Hcr^+ thy^- trp^- were irradiated by a given dose of UV, all thymine dimers were subsequently excised, leaving no detectable traces. If, however, DNA synthesis before irradiation was inhibited either by thymine starvation or by the enrichment of medium by cytidine [5], the capacity of cells to excise dimer was considerably lowered (fig. 1).

It is very well known that thymine starvation leads to thymineless death [6] and exhibits a sensitizing effect to UV [7]. Thus the lowered capacity for excision in the cells pretreated without thymine could be interpreted as a consequence of the loss of viability.

As described, the negative effect of thymine starvation on the viability [8] and UV sensitivity of cells [7] can be prevented by a simultaneous inhibition of protein and RNA syntheses. Applying this intervention, however, we could not find any restoration of the capacity of cells to excise dimers (fig. 2).

Our data indicate that the excision of dimers can be considerably influenced by the pre-starvation of cells for thymine. The fate of unexcised dimers after transferring the cells to plates and the possible excision of dimers in longer pieces (which would be precipitable in trichloroacetic acid thus simulating unexcised dimers) is under investigation.

References

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