EFFECT OF AMINO ACID STARVATION ON THE DEGRADATION OF DNA IN *ESCHERICHIA COLI* B/r AFTER UV IRRADIATION

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1. Introduction

Resistance of bacterial cells to UV irradiation can be considerably influenced by the inhibition of protein synthesis during their preirradiation growth [1]. The inhibition of protein synthesis, for example by omitting essential amino acids, results in a delay of DNA synthesis and a delay of cell division after UV irradiation. Thus the cells are given more time for repair of damaged genetic material prior to its replication, so that before its replication the UV damaged DNA molecule is more or less repaired [2]. As was shown later, a condition for the increase of bacterial UV resistance is that the cells are capable of repair; the UV sensitive *E. coli* B/s_1 mutant, containing both *exr*$^-$ and *hcr*$^-$ mutations does not display any increase of resistance after amino acid prestarvation [3]. From this it is possible to conclude that the pretreatment in some way affects the repair processes.

It was found that the increase of UV resistance after amino acid prestarvation does not result in increase of the repair synthesis [4]. Similarly no direct relationship was found between increase of UV resistance following amino acid prestarvation and excision of pyrimidine dimers [5]. These findings led to consideration of the size of the gaps after the excision of dimers. It was postulated that the activity of exonucleases after prestarvation could be partially inhibited, so that the gaps, after dimer excision, would be smaller than in logarithmic cells. Therefore, the same amount of repair synthesis would be sufficient to repair a greater number of gaps [4].

To test this hypothesis, we compared release of radioactivity from UV irradiated DNA prelabelled with thymine-2-^{14}C and the level of thymine-2-^{14}C released into medium in logarithmically growing and amino acid prestarved cells.

2. Materials and methods

E. coli B/r thymine$^-$ tryptophan$^-$ *hrc*$^+$ were used in these experiments. Cells were grown in mineral salt medium with 1% glucose supplemented with 2 μg of thymine and 14 μg of tryptophan per ml at 37° to about 1×10^8 cells/ml. For DNA labeling, thymine-2-^{14}C (0.2 μCi/ml, sp. activity 44 mCi/mmole) was used.

At the early logarithmic phase of growth (density given above) cells were harvested by membrane filtration, washed and resuspended in complete medium without added radioactive thymine and incubated for 30 min to use up labelled thymine from the metabolic pool. After repeated filtration, the cells were suspended in the medium lacking tryptophan. Tryptophan was added to an aliquot of the suspension and the cells were irradiated with UV (750 ergs/mm^2, TUV 15 Philips Germicidal lamp). A second portion of the suspension, starved of tryptophan, was incubated for 120 min and then irradiated as mentioned above. Both logarithmically growing and prestarved cells were incubated for a period after irradiation.

UV resistance: The number of surviving cells was

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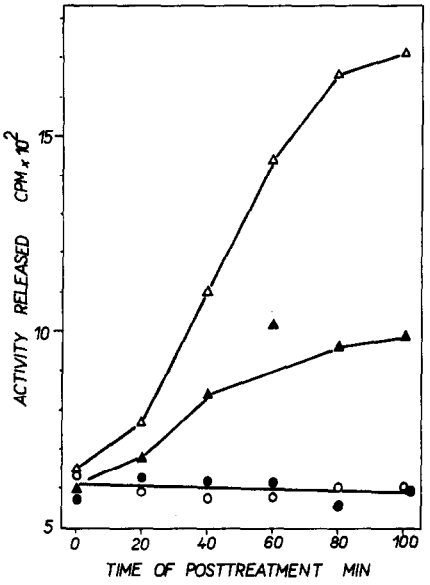

Fig. 1 Releasing of radioactivity of thymine-2-^{14}C from cells into medium following aminoacid prestarvation and UV irradiation in *E. coli* B/r thymine$^-$ tryptophan$^-$ *hcr*$^+$,
o—o logarithmic control without UV;
Δ—Δ logarithmic control irradiated with 750 ergs/mm^2;
●—● prestarved culture without UV;
▲—▲ prestarved culture irradiated with 750 ergs/mm^2.

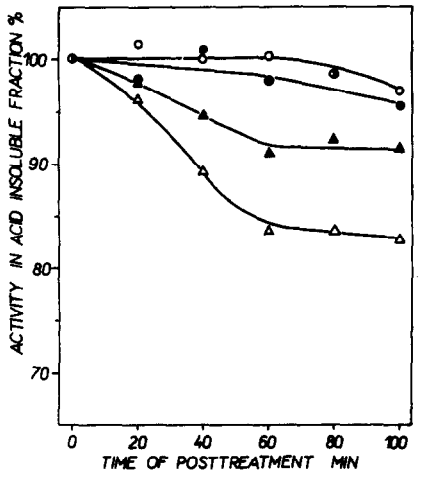

Fig. 2. Decrease of radioactivity of thymine-2-^{14}C in acid insoluble fraction of *E. coli* B/r thymine$^-$ tryptophan$^-$ *hcr*$^+$ cells after amino acid prestarvation and UV irradiation,
o—o logarithmic control without UV;
Δ—Δ logarithmic control irradiated with 750 ergs/mm^2;
●—● prestarved culture without UV;
▲—▲ prestarved culture irradiated with 750 ergs/mm^2.

determined at the beginning and end of the period of starvation by suitable dilution of irradiated cells and incubation on the solid synthetic medium described above, containing 1.5% agar. The surviving fraction is expressed as the number of surviving irradiated cells divided by the number of non irradiated cells after 24 hr cultivation at 37°.

Degradation of DNA: Samples (1 ml) of bacterial suspension were withdrawn periodically after irradiation into ice cold TCA to give a final concentration of TCA of 5%.

For determination of radioactivity in the supernatant fraction, samples were centrifuged at 30,000 *g* for 30 min and aliquots of supernatants counted in a dioxan liquid scintillator in a Packard Tricarb Spectrometer 3375.

For the determination of radioactivity in acid insoluble fractions, the samples were taken in the same way and filtered on membrane filters (porosity 0.4 μm). Dried filters were placed in vials with toluene scintillator for counting.

3. Results and discussion

The increase of UV resistance for *E. coli* B/r during 120 min tryptophan starvation is estimated by colony formation. The surviving fraction at the beginning of the starvation period was 3.36×10^{-3} and at the end was 7.92×10^{-1}; the resistance was increased 236 times. The amount of DNA degradation after UV irradiation of prestarved cultures was shown to be significantly different compared with degradation observed in logarithmically growing cells by both methods of determination (figs. 1 and 2).

It is possible that prestarvation decreases the activity of exonucleases. The difference in the extent of degradation could explain the increased survival following prestarvation with the same extent of excision of dimers and the same or even smaller repair synthesis. It is necessary to postulate that exonucleases after amino acid prestarvation could produce smaller gaps which are easier to repair. The smaller repair synthesis is then completely reasonable, since for repair of smaller gap a smaller number of nucleotides is necessary.

The above interpretation is based on the assump-

tion that degradation after UV irradiation is a step of the "excision repair" process. This assumption is in agreement with the finding that DNA degradation takes place in UV irradiated cells, capable of excision of dimers and that it was not found in cells, which do not possess this capability [6].

However, direct proof is not available that the observed extensive degradation is an inevitable step in the repair process. It cannot be ruled out that DNA degradation of non-surviving cells occurs which started the repair process by excision of dimers, but are unable to complete successfully further repair steps [7].

A definitive explanation for lower postirradiation DNA degradation after prestarvation for amino acids and its potential importance for the increase of UV resistance of cells will therefore require further study.

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