POST-IRRADIATION DEGRADATION OF DNA FOLLOWING EXPOSURE OF UV-SENSITIVE AND RESISTANT BACTERIA TO X-RAYS

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It has been discovered that certain bacteria, including E. coli, are able to rid themselves of thymine dimers formed by ultraviolet light in their DNA. In this repair process, the dimers are removed or excised from the DNA in the form of a fragment, sometimes recovered as a trinucleotide containing part of the phosphodiester backbone. Cells able to carry out this repair undergo post-irradiation degradation of the DNA manifested as a release of nucleotides into the acid-soluble fraction of the cell and into the surrounding medium after irradiation (Setlow and Carrier, 1964; Boyce and Howard-Flanders, 1964). Neither thymine dimer excision nor UV-induced breakdown occurs in UV-sensitive strains of E. coli that have suffered a mutation at any one of the uvr loci and that are unable to repair UV-irradiated T1 phage DNA (Howard-Flanders, Boyce and Theriot, in preparation).

Removal of the DNA fragment containing the thymine dimer from ultraviolet resistant uvr^+ cells is presumably due to the action of an endonuclease, specific for certain defects in the DNA and which is inactive or missing in the uvr mutants. There are two possible explanations for the ensuing release of nucleotides. This defect excision endonuclease may have an additional exonuclease activity that is responsible for the DNA breakdown. If so, the ability to degrade DNA might be lacking in certain or all of the uvr mutants.

Alternatively, a different enzyme may be responsible for the breakdown and will remain active in the uvr mutants, the lack of breakdown after exposure to UV light being simply due to the absence

of gaps left by thymine dimer excision in which the exonuclease can start breakdown. It seemed possible to distinguish between these two alternatives by exposing these mutants to X-rays, also known to cause DNA breakdown in wild type E. coli (Stuy, 1960; Drakulic and Kos, 1963). If X-ray induced DNA breakdown does not occur in the strains carrying mutations at any of the three loci uvrA, uvrB and uvrC, known to control thymine dimer excision, then the endonuclease for thymine dimer excision must also degrade DNA.

In previous investigations of post X-irradiation breakdown of DNA, the relationship between extent of degradation and dose was not determined. A study of this was included in the present work.

Materials and Methods

The genetic characteristics (Howard-Flanders, Boyce, Simson and Theriot, 1962; Howard-Flanders, Boyce and Theriot, in preparation.) of the four bacterial strains used are shown in Table I.

TABLE I. Characters of the E. coli K-12 F^- Strains

Strain	uvr*	Nutritional Requirements						Energy Source Utilization					Phage Growth			Drug Res.
		Threonine	Leucine	Proline	Histidine	Thiamine	Arginine	Lactose	Galactose	Arabinose	Xylose	Manitol	T1	T6	λ	Streptomycin
AB1157	+	-	-	-	-	-	-	-	-	-	-	-	S	R	S	R
AB1884	C-34	-	-	-	-	-	-	-	-	-	-	-	S	R	S	R
AB1885	B-5	-	-	-	-	-	-	-	-	-	-	-	S	R	S	R
AB1886	A-6	-	-	-	-	-	-	-	-	-	-	-	S	R	S	R

* uvrA, uvrB and uvrC are used to denote the mutant loci in cells that are unable to repair UV-irradiated T1 bacteriophage.

The bacteria were grown with aeration at 37°C to early stationary phase in M9 medium (containing per liter of water: 1 g NH_4Cl, 11 g $Na_2HPO_4 \cdot 7H_2O$, 3 g NaH_2PO_4, 5 g NaCl, 120 mg $MgSO_4$, 4 g glucose), supplemented with 2.5 mg/ml casamino acids, 0.5 μg/ml thymine, 250

μg/ml deoxyadenosine to increase the incorporation of thymidine (Boyce and Setlow, 1962), and 0.2 μg/ml H^3-TdR of specific activity 15C/mM. Grown cells were washed twice in M9, resuspended in M9 and incubated with aeration at 37°C for 1 hour to use up labeled thymidine from the metabolic pool. After washing once more in M9, they were suspended in buffered saline (containing 2.68 g Na_2HPO_4 . 7 H_2O, 1.36 g KH_2PO_4 and 7.6 g NaCl per liter of water) and each strain was irradiated with X-rays (250 kV, 15 mA; Dose rate = 8.5 kilorads/min) at 0°C in the presence of oxygen.

The irradiated suspensions and unirradiated controls were incubated with aeration at 37°C, and at suitable time intervals samples were withdrawn, centrifuged, and aliquots of the supernatant assayed for radioactivity using a liquid scintillation counter.

Results

Figure 1 shows a graph of radioactivity released into the medium,

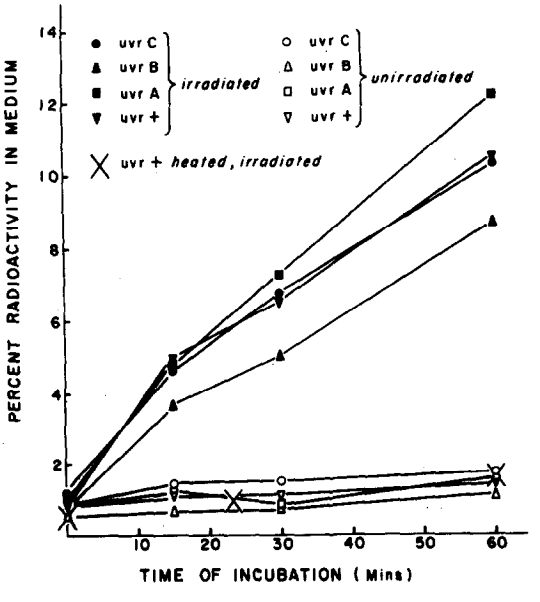

Figure 1. Release of radioactivity into the medium from the DNA of E. coli K-12 labeled by growth in medium containing tritiated thymidine. The strains uvrA, B and C and uvr^+ were irradiated with 21 kilorads of X-rays, incubated with aeration at 37°C, and the degradation measured by appearance of radioactivity into the medium. Results obtained with unirradiated controls and with uvr^+ heated at 65°C for 15 mins. prior to irradiation are also shown.

expressed as a percentage of total activity, plotted against time of post-irradiation incubation at 37°C for the four strains. It appears that extensive degradation occurs in all four strains and at approximately the same rate. Control samples heated for 15 minutes at 65°C before irradiation and unirradiated samples show negligable post-irradiation degradation. Therefore degradation occurs only after irradiation and is probably due to a heat sensitive enzyme.

Some experiments were carried out to relate post-irradiation degradation to dose received and to survival of reproductive capacity. Figure 2 shows the way the rate of release of activity into the medium varies with dose for the uvr$^+$ strain. It can be seen that the extent of

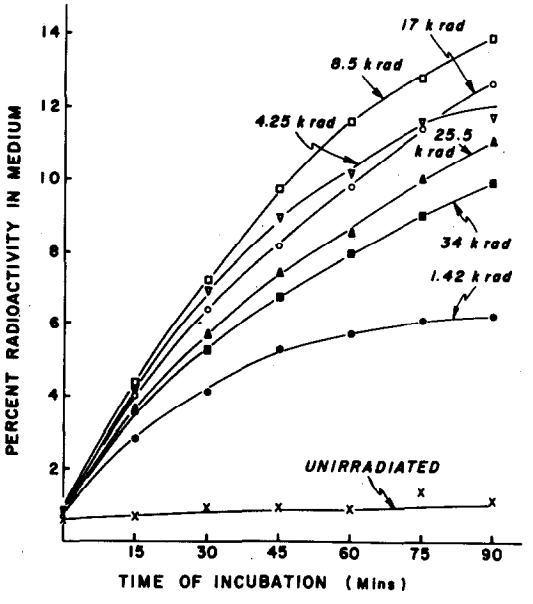

Figure 2. Release of radioactivity into the medium from the DNA of E. coli K-12 uvr$^+$ as a function of X-ray dose and of time of post-irradiation incubation with aeration at 37°C.

post-irradiation degradation goes through a maximum at a dose of about 8.5 kilorads, and a dose of only 1.5 kilorads is sufficient to give half of this amount of degradation. By plating on nutrient agar, it was found that 1.5 kilorads left about 70% survivors and 8.5 kilorads about 50%.

Discussion

The results show that the uvrA, B and C mutants are capable of

breaking down their DNA after X-irradiation. Therefore, the enzymes for defect excision which are controlled by these loci are not responsible for the degradation of the DNA, which must, therefore, be the work of other enzymes. All four strains must possess the enzyme(s) necessary for degradation. Moreover, all four strains must either possess excision enzymes for ionizing radiation products or, less likely, excision enzymes are unnecessary in the case of ionizing radiation due perhaps to the greater likelihood of radiation induced chain breaks.

Post irradiation degradation occurs after low X-ray doses. Only 1.5 kilorads are required for half of the maximum rate of degradation. This dose, which kills about 30% of the bacteria will produce about 12 ionization in a DNA of 2×10^9 M. Wt. (Cairns, 1963). This estimate is based on the assumption that 25 eV are required per ionizing event and neglects indirect action of radiation produced free radicals from the cellular water, which could cause additional changes and increase the number of products by an unknown factor, possibly between 1 and 5.

At about 9 kilorads the extent of degradation goes through a maximum and thereafter decreases. This could be caused by the greater number of radiation products or chain breaks produced by higher doses leaving shorter lengths of undamaged DNA upon which the enzymes responsible for degradation can act.

The results also show that 6% of the DNA breaks down after exposure to 1.5 kilorads of radiation. This corresponds to the release of 3.6×10^5 nucleotides for a dose that probably produces between 12 and 60 radiation products in the DNA. Thus, at least 6×10^3 nucleotides are released per estimated radiation product. Although the basis of this calculation is in doubt by a small factor, it shows the process of post-irradiation degradation to be remarkably efficient. In view of the association of breakdown after UV irradiation with DNA repair it seems possible that the breakdown after X-rays may also reflect the action of a repair process involving breakdown and reconstruction of the DNA.

In summary, it is concluded that in E. coli K-12, the capacity to degrade DNA after X-irradiation is not affected by a mutation at any one of the genetic loci uvrA, uvrB or uvrC, which are known to control

thymine dimer excision. At low doses it was found that several thousand nucleotides are released per estimated radiation product formed in the DNA.

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