RADIATION PRODUCES BREAKS IN L CELL AND MOUSE LIVER DNA CHARACTERIZED BY 5' PHOSPHORYL TERMINI

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SUMMARY

Using the polynucleotide kinase method, we were able to demonstrate the repair of radiation induced DNA breaks in mouse liver DNA and in L cells. The specificity of the enzyme indicates the breaks to have 5' phosphoryl termini.

INTRODUCTION

Richardson recently described an enzyme, polynucleotide kinase, which in the presence of gamma-labeled AT^{32}P, labels DNA breaks which have 5' phosphoryl termini (Richardson, 1965, 1966). In this communication we report results of experiments in which L cell DNA and mouse liver DNA were irradiated in vivo, extracted, and then reacted in the polynucleotide kinase-AT^{32}P system. We found that radiation produced breaks, characterized by 5' phosphoryl termini, in both types of DNA and that these breaks were rapidly repaired.

METHODS

The L cells were grown and handled as described (Dalrymple, Sanders, and Baker, 1967). Immediately before irradiation (250 kVp x-rays; 400 rads/min) the cells were detached from the culture bottles with a dilute trypsin solution, washed twice with fresh medium (Eagle's MEM), and then suspended at a concentration of 5×10^6 cells/ml. For each irradiation point paired 3 ml samples were collected; one sample was irradiated while the other served as a control.

After collection the cells were centrifuged in the cold; the cell pellet

was washed twice with ice cold saline-citrate (0.15 M NaCl--0.015 M Sodium Citrate) and then suspended in 1 ml of saline-citrate. Three drops of 1% sodium dodecyl sulfate were added to lyse the cells. The RNA and protein were removed by the procedure described by Cleaver (1968). The DNA content was measured by the diphenylamine method (Burton, 1968). The gamma-labeled $AT^{32}P$ was prepared according to the method described by Glynn and Chappell (1964).

The polynucleotide kinase was extracted, purified (through step IV), and reacted with the L cell DNA according to methods described by Richardson (1966). Briefly, this technique requires incubation of the DNA with E. coli alkaline phosphatase (Worthington Biochemicals) after which it is incubated with the enzyme and $AT^{32}P$. Within a given experiment, equal amounts of polynucleotide kinase and $AT^{32}P$ were used for each sample. Also, the amounts of DNA in each sample were essentially equal. At the end of the last incubation period, perchloric acid was added to a final concentration of 0.5 N and the acid insoluble material collected on millipore filter discs. The discs were then washed with additional 0.5 N perchloric acid and allowed to dry. Radioactivity was measured with a liquid scintillation counter.

Similar steps were followed for the experiments with mice. Young adult Swiss Webster mice of either sex were used. They were held in a lucite chamber during irradiation (100 rads/min); the exposures were total body. At the appropriate time after irradiation the animals were sacrificed by cervical fracture, and the livers rapidly removed. The tissue was placed on ice and cut into small pieces. Approximately 30 mg of liver was homogenized in 1 ml of saline-citrate. After this, 1 ml of 1% dodecyl sulfate was added and the homogenization continued, until the solution became clear and quite viscid (due to the release of the DNA). The tubes were centrifuged to remove the small amount of cellular debris, and the supernatant was treated, as before, to remove RNA and protein. Following dialysis, the optical density of the solution (260 mμ) was adjusted to 1.0; a portion was taken for measurement of DNA. Additional portions were taken for the polynucleotide kinase reaction. The samples were precipitated, collected on filter discs, and counted as described above.

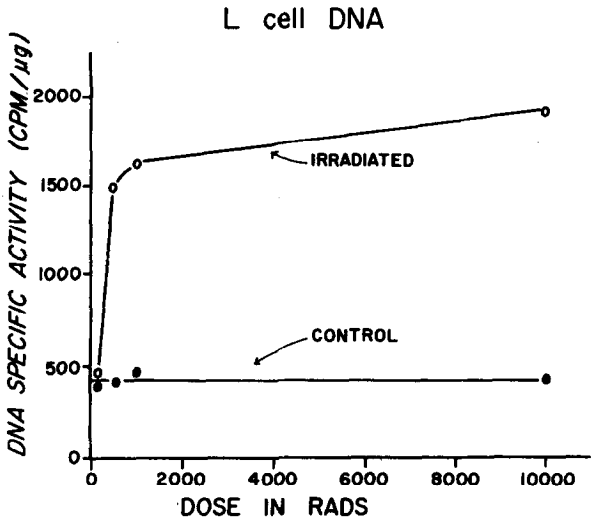

Fig. 1. Labeling of 5' phosphoryl termini of L cell DNA after different doses of radiation. The ordinate has units of cpm/μg DNA.

RESULTS AND DISCUSSION

Fig. 1 shows the effect of 100 to 10,000 rads on L cell DNA. For this experiment the samples were irradiated and lysed as soon as possible after the exposure. Notice that the specific activity (cpm/μg DNA) of the incorporated $^{32}PO_4$ increases rapidly until 500 rads, after which the rate of incorporation decreases. Recall that increased labeling indicates an increased number of 5' phosphoryl termini. Clearly the 500, 1000, and 10,000 rad points fall well above control. A similar response pattern was found by Lohman (1968), who used ultracentrifuge methods.

Fig. 2 shows the effect of 500 rads on L cell DNA. For this experiment, the cells were irradiated and then lysed at various time points after exposure. Notice that the amount of incorporated radioactivity decreases with time after irradiation until a constant value is reached. Since the decreasing count rate indicates a decreasing number of 5' phosphoryl termini, we assume this to represent repair of these "lesions." Using a semi-log plot (not shown), the first subtraction exponential indicates a repair half-time of approximately 1 minute.

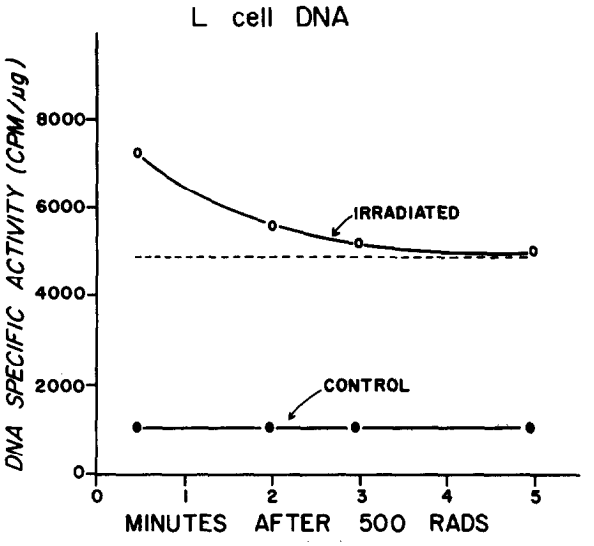

Fig. 2. Labeling of 5' phosphoryl termini of L cell DNA, as a function of time after 500 rads.

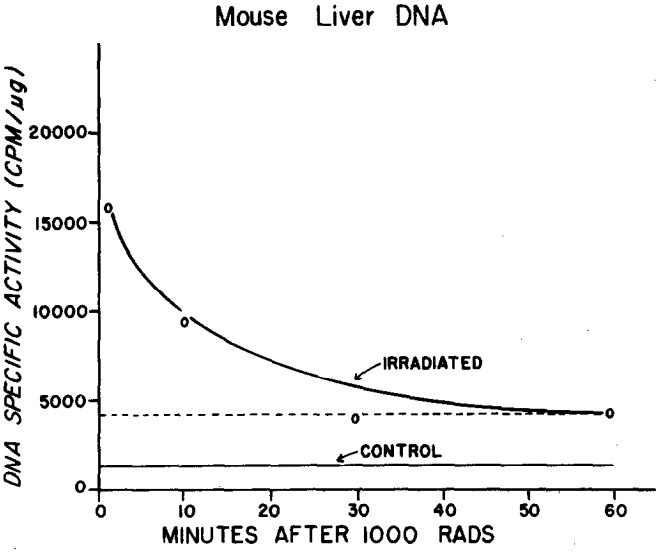

Fig. 3. Labeling of 5' phosphoryl termini of mouse liver DNA, as a function of time after a total body dose of 1000 rads.

Fig. 3 shows the effect of 1000 rads on mouse liver DNA. As in the case of Fig. 2, the animals were irradiated and the livers removed at the time points shown. Although the half-times differ somewhat, notice the similarity in the pattern of incorporation of the $^{32}PO_4$ into mouse liver DNA as compared with the L cell DNA. A semi-log plot indicates a repair half-time of about 10 minutes.

The results of our experiments show DNA breaks produced by relatively low doses of radiation to be easily identifiable with polynucleotide kinase. Because of the specificity of this enzyme, these breaks, then, should have 5' phosphoryl termini. Also, since the breaks are rapidly repaired after irradiation, the breaks should be single stranded. The asymptote approached by irradiated DNA (for both L cell and liver) indicates more 5' phosphoryl termini than are present in the controls. Consequently, we are lead to the conclusion that the difference between controls and this asymptote represents breaks which are irreparable. These breaks, then, should be double stranded, while those that were repaired should be single stranded.

The repair half-times observed by the polynucleotide kinase assay agree with ultracentrifuge studies in our laboratory and in others (Lohman, 1968). The difference between the half-times for the repair of DNA breaks and the "repair" half-times found with paired-dose survival experiments (about 1.7 hrs. for our L cells), while very interesting, cannot be explained as yet (Dalrymple, Sanders, Baker, and Wilkinson, 1969).

During the last few years several workers have isolated an enzyme--DNA ligase--from E. coli and several mammalian tissues (Weiss and Richardson, 1967; Lindahl and Edelman, 1968). This enzyme catalyzes the closure of single stranded DNA breaks characterized by 5' phosphoryl, 3' hydroxyl termini. As a result, our experience would suggest DNA ligase to be active and to be responsible for the repair of the single stranded breaks in DNA. Additional work is in progress in our laboratory to further evaluate this conclusion.

A recent paper by Kapp and Smith (1969) describes experiments which would seem to be at variance with our experience. These authors were unable to demon-

strate rejoining E. coli DNA by E. coli polynucleotide joining enzyme in an in vitro system. From their results they concluded that most of the breaks (greater than 66%) were not characterized by 5' phosphoryl termini. Because the experimental techniques were considerably different than ours, and since their experiments were totally in vitro, perhaps one should not expect similar results. Also, as these authors point out, other types of breaks are certainly possible. While our results would indicate some breaks contain 5' phosphoryl termini, we certainly do not claim that all breaks are of this type.

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