RADIATION ALTERS THE ABILITY OF MAMMALIAN CELL DNA TO COMPETE IN A DNA:DNA HYBRIDIZATION SYSTEM

Glenn V. Dalrymple, J. L. Sanders, A. J. Moss, Jr., Max L. Baker, J. C. Nash, and K. P. Wilkinson

Division of Nuclear Medicine and Radiation Biology
Department of Radiology, the Division of Biometry, and the
Department of Physiology and Biophysics
The University of Arkansas Medical Center
and
The Nuclear Medicine Service
Veterans Administration Hospital
Little Rock, Arkansas 72206

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SUMMARY

Five thousand rads of X-rays destroyed the ability of L5178Y DNA to compete in a DNA:DNA hybridization system.

INTRODUCTION

Ionizing radiation causes a spectrum of damage to DNA ranging from scissions in the phosphodiester backbone to base damage (Kanazir, 1969). Of the many types of damage demonstrated, the actual nature of the biologically significant radiation lesion, as it occurs in vivo, still eludes description. During the past five years, considerable information has emerged about the repair of radiation damage to the phosphodiester backbone (Kaplan, 1966).

The question of radiation induced base damage, in vivo, has attracted considerably less attention because of the lack of suitable experimental techniques. This is especially true for mammalian cell DNA. The work of Goddard, Weiss, and Wheeler suggested that γ radiation caused base damage to ribonucleotide homopolymers such that misreading occurred in an RNA-RNA polymerase system (Goddard, et al., 1970). Their experience suggested to us that radiation could produce some form of damage which would prevent the

formation of duplexes with non-irradiated complementary DNA. This report describes the results of experiments which support this possibility.

METHODS

L5178Y cells (murine lymphoblastic lymphoma) were maintained both as ascites tumors (in BDF mice) and in cell culture. The cultured cells were grown in Fisher's medium supplemented with 10% horse serum. The cultured cell DNA was labeled by adding 2 μCi/ml of tritiated thymidine (27.8 Ci/mMole; Amersham/Searle) for 2 days prior to harvest of the cells.

The DNA was isolated from the cells and purified according to the method described by Moss, et al., (1971). The DNA was then further purified by hydroxyapatite (HA) chromatography (Bernardi, 1971); Fig. 1 shows the chromatogram for the ^{3}H labeled DNA used in this report.

One mouse with an ascites tumor was irradiated with 5000 rads of X-rays (250 kVp, 200 rads/min, HVL 2mm Cu) and then sacrificed by cervical fracture

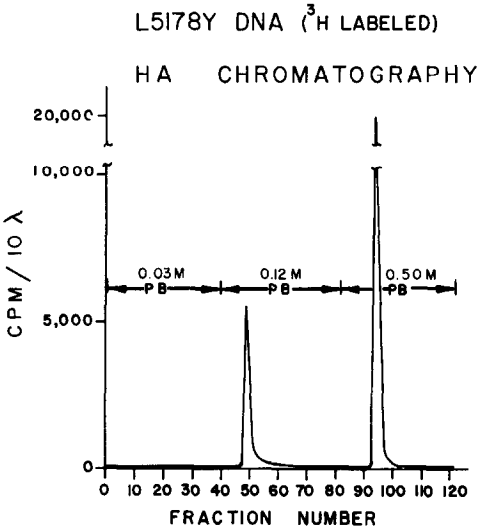

Fig. 1. Hydroxyapatite chromatogram of ^{3}H labeled DNA. The purified DNA was loaded onto a hydroxyapatite (HA) column (Biorad). The HA had been prepared according to the method of Tiselius (Bernardi, 1971). The column was eluted by step-wise elution with Na phosphate buffer pH 6.8 (PB). A 10 λ portion of each fraction (2 ml) was counted. Fractions 48-52 were pooled and dialyzed for this study. Thermal denaturation profiles showed the DNA eluted at 0.12 M PB to be single stranded, while the DNA eluted at 0.50 M PB was double stranded.

immediately after exposure. A sham irradiated control was sacrificed at the same time. The cells were removed immediately from the animals and washed with Hank's balanced salts solution (HBSS). The DNA from both control and irradiated cells was purified in the same manner as the cultured cell DNA--including HA chromatography. The DNA from the 0.12 M Na phosphate buffer, pH 6.8 (PB) eluents were pooled and dialyzed against 6XSSC (1XSSC = 0.15 M NaCl--0.015 M Na citrate). The DNA prepared in this manner is single stranded. The DNA was analyzed by sucrose gradient sedimentation; the weight-average molecular weight was approximately 0.5 - 1.0 x 10^6 Daltons (Moss, et al., 1971). In addition, the DNA eluted at 0.12 M PB was acid insoluble and diphenylamine positive. Consequently, we are confident that 0.12 M PB eluent is truly DNA and not a mixture of oligonucleotides. In our experience the HA chromatography of the DNA is necessary to provide consistent and reproducible results.

Nitrocellulose membrane filters (25 mm, 0.45 μ, Millipore Corp.) were prepared by the method of Denhard (1966) and then loaded with non-labeled HA chromatographed cultured cell DNA. Each filter contained 11 ± 1 (S.E.) μg of DNA.* Groups of five filters each were incubated for 16 hours at 60° with increasing concentrations (0 to 20 μM DNA-P) of either control or irradiated DNA.

The DNA was dissolved in 6XSSC; the incubation volume was 1 ml. The DNA in solution will be termed the competitor DNA. Following incubation the competitor DNA was removed by aspiration and the filters washed with several changes of 6XSSC. One ml of the ^3H labeled single strand DNA was then added (60 μM DNA-P; 36,000 cpm/ml) and the filters incubated for another 16-hour period. The filters were then washed, as described by Denhard (1966), dried, and counted with a Beckman LS200 liquid scintillation counter.

RESULTS AND DISCUSSION

Fig. 2 shows the results of the experiment. The figure shows the

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* The amount of DNA on the discs was determined in parallel experiments using ^3H labeled single stranded DNA to load the discs.

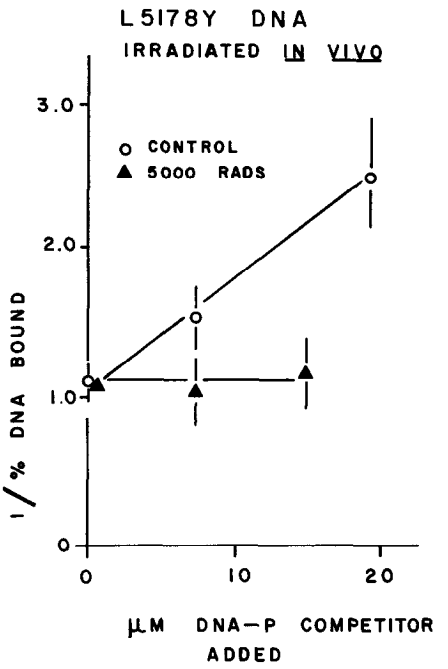

Fig. 2, Change in ability of DNA to compete after irradiation. The figure shows the reciprocal of the % labeled DNA bound as a function of competitor DNA concentration; the points are average of five filters. The uncertainties are standard errors. The figure shows 5000 rads to destroy the ability of L5178Y DNA to compete in this DNA:DNA hybridization system.

reciprocal of the % labeled DNA bound to the DNA on the filters as a function of competitor DNA concentration (Dixon and Webb, 1958). The uncertainties are std errors. As the figure indicates, the control DNA competed very effectively with the ^{3}H labeled DNA for binding sites on the filter DNA. We have also found that DNA extracted from solid and ascites tumors competes as effectively as DNA from the L5178Y cells in culture. Irradiated DNA, however, was not able to compete. In other experiments (not shown) we have observed similar findings for doses from 10,000 to 200,000 rads. In the other direction, we have studied doses as low as 500 rads. Preliminary results suggest that 500 rads probably represent the lowest dose at which inhibition of annealing can be detected. Additional dose-response studies, together with time-dose experiments are in process in our laboratory.

Our results suggest that, functionally, regions of the irradiated DNA molecule are not able to recognize complementary regions of non-irradiated molecules. This may mean that some form of base damage could be present; simple breaks in the phosphodiester backbone produced by mechanical shearing do not seem to alter annealing properties of DNA (Britten and Kohne, 1968). We cannot exclude, however, the possibility that damage to deoxyribose moieties could play a role.

The weight-average molecular weight of our purified DNA (0.5 - 10 x 10^6 Daltons) is considerably lower than values reported for native mammalian cell DNA. Also, there was no difference in size of our irradiated DNA as compared with control. Consequently, although the preparation and purification of the DNA reduces the molecular weight, it does not prevent control DNA from competing in our DNA:DNA hybridization system. These findings agree with the experience of Britten and Kohne (1968) who used mechanical shearing to reduce the size of the DNA used for their annealing studies.

The biologic importance of our findings cannot be assessed at this time. Possibly the function of DNA in transcription and replication could be sufficiently altered to cause cell lethality. Additional studies are in process in our laboratory to contrast postirradiation cell lethality and the repair of single strand DNA breaks with changes in ability of DNA to hybridize.

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