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IS THERE A RELATIONSHIP BETWEEN NUCLEOTIDE SEQUENCE AND RADIATION INDUCED DNA DAMAGE ?

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ABSTRACT

Plasmid DNA in aqueous solution were exposed to 30 to 240 Gy of ^{60}Co γ rays. It caused dose dependent single stranded breaks in the DNA. The irradiated plasmids were separately restricted by endonuclease *Hinf I, Hae II* and *Bgl I*. The DNA fragments generated by *Hae II* restriction of unirradiated and irradiated plasmids showed significant differences in their migration. In cases of *Hinf* and *Bgl I* such differences were not observed. The large size fragments produced by *Hae II* restriction of γ ray irradiated plasmid indicates that restriction was inefficient and partial. This may be possible if the *Hae II* restriction site (A/GGCGC↓T/C) or its flanking nucleotide sequence(s) were modified by radiation making the sequence(s) unavailable for complete and normal restriction. Since the restriction sites for *Hinf I* and *Bgl I* are qualitative different from that of *Hae II*, it appears likely that GC-rich nucleotide sequences are vulnerable to radiation induced damage(s).

INTRODUCTION

It is accepted that ionizing radiation causes non-random biological effects; the eventual biological effect of a cell being dependent on its radiosensitivity. However, the molecular basis of the variable inherent radiosensitivity in living system is not well understood. Radiosensitivity of a cell is mostly defined in relation to their ability to repair the wide spectrum of DNA damage (1). The structural organization of the DNA has been shown to play a role in the radiosensitivity of cells depending on chromatin compactness (2). In contrast to actively transcribing genes being more sensitive due to partial decondensation (3,4), non-transcribing genes show equally high radiosensitivity (5). This suggests that structural organization of DNA may not be the only factor influencing the radiosensitivity. Mutation studies have also suggested that the proximate nucleotide (NT) sequence and organization of DNA are important in the production of DNA damage (6). In an attempt to understand the variable inherent radiosensitivity, genetic instability and genetic predisposition, we have studied the relationship between NT sequence and radiation-induced DNA damage in a plasmid DNA model.

MATERIALS AND METHOD

Preparation of plasmid: *E.coli* XL 1 blue strain was grown overnight in an ampicillin selection LB medium at 37 °C. Its plasmid, the blue vector incorporating a known DNA fragment, HBVPREX, was extracted from the cells by standard miniprep protocol with minor modifications (7). The plasmid was dissolved in sterile distilled water. Ethanol precipitation was repeated after RNase treatment.

Radiation and irradiation condition: Aqueous plasmid DNA preparation (41.25 μg ml^{-1}) was irradiated in an Eppendorf tube in a ^{60}Co gamma chamber (~0.2 Gy sec^{-1}) to accumulate doses of ~30, 60, 120, and 240 Gy.

Restriction fragmentation and analysis: Irradiated and unirradiated (control) plasmids were analyzed by 1% agarose gel electrophoresis in 40 mM tris-acetate buffer containing 1 mM

EDTA. Gels were stained in 0.3 μg ml^{-1} ethidium bromide for 10 min. The plasmids were separately subjected to *Hinf I*, *Hae II* and *Bgl I* endonuclease restriction. Restriction conditions were as per manufacturers recommendations (Genei, India) and the fragments were resolved by agarose gel electrophoresis.

RESULTS AND DISCUSSIONS

Fig. 1 shows the three forms of plasmid DNA in control and following irradiation. As evident, there were dose dependent changes in two of the three forms of the plasmid. Densitometric quantification showed dose dependent reduction in the quantity of closed circular (CC) form 4, 7, 11 to 16% after doses of 30, 60, 120 and 240 Gy, respectively. Simultaneously, there was dose dependent increase in the quantity of open circular (OC) form from 3, 6, 10 to 14%. The linearized (L) form, however, showed negligible increase of about 1% at the highest dose of 240 Gy.

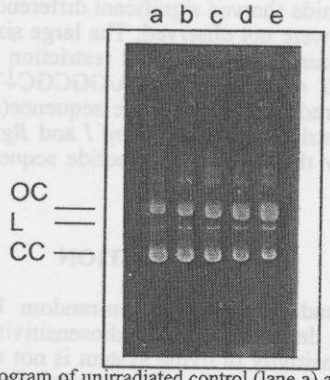

Figure 1: Agarose gel electropherogram of unirradiated control (lane a) and γ irradiated plasmid DNA (lanes b, c, d and e corresponding to doses of 30, 60, 120, and 240 Gy, respectively); CC = closed circular, OC = open circular and L = linearized forms of the plasmid.

Fig. 2 shows the results of restriction fragment pattern of plasmid generated by *Hinf I* (G↓ANTC), *Hae II* (A/GGCGC↓T/C) and *Bgl I* (GCCNNNN↓NGGC). In *Hinf I* (Fig. 2 A) and *Bgl I* (Fig. 2 C) restricted plasmid, there was no observable changes in its restriction patterns for unirradiated and irradiation samples. In contrast, the irradiated plasmid restricted with *Hae II* showed slow migrating bands or large DNA fragments on the gel which were entirely absent in the unirradiated control. The observed change was observed in all irradiation groups. Presently we are unable to explain this radiation dose independent observation (Fig: 2 B).

The results show variations in restriction capabilities of endonucleases after plasmid DNA has been exposed to γ rays. Radiation dose dependent significant decrease in CC and increase in OC forms and insignificant increase in L form of plasmid (Fig. 1) indicates that γ radiation induced primarily single strand breaks (SSBs). The SSBs seem not to affect the restriction sequences as restriction fragmentation by *Hinf I* and *Bgl I* remained unchanged after irradiation (Fig. 2 A & C). On the other hand, the restriction sequence for *Hae II* was influenced by radiation. This led to partial fragmentation of the irradiated plasmid (Fig. 2 B). The pattern suggests that some modification in the restriction sequence has made the plasmid resistant to *Hae II*. Incomplete cutting has, therefore, produced larger DNA fragments which is seen in the gel as slow migrating bands. It is interesting to note that all three restriction endonucleases used in this investigation produce sticky ended fragments (Table I). While the terminal single stranded end is -GCGC in case of *Hae II*, those for *Hinf I and Bgl I* are -TT/AA and –TTT, respectively (Table I).

A closer look reveals that only GC or GC-rich restriction sequence was effected by radiation such that restriction ability was reduced (Fig. 2 B). The AT-rich restriction sequence was

unaffected by radiation (Fig. 2 A & C). This points to NT sequence specific effect of radiation on DNA. The present investigation points to this fact in the realm of restriction sequence only.

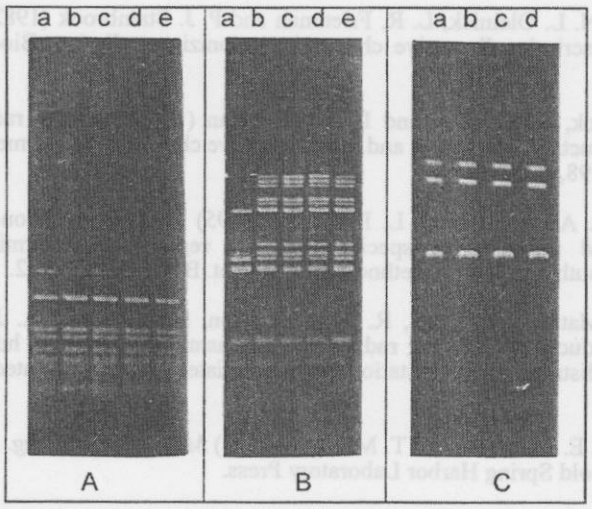

Figure 2: Restriction fragment analysis on 1% agarose gel electrophoresis. Lanes a, b, c, d and e show unirradiated control, 30, 60, 120 and 240 Gy γ irradiated plasmid, respectively, restricted by different enzymes; panel A = *Hinf I*, panel B = *Hae II* and panel C = *Bgl I*.

However, the involvement of neighboring or flanking NT sequence(s) in this effect can not be ruled out. More work is under progress. The implications of this preliminary finding are of possible importance in the assessment of radiosensitivity, genetic predisposition and genetic instability. The results suggest that GC-rich NT sequences are more vulnerable to radiation

Table I: Nucleotide sequences of restriction sites on HBVPREX and fragment generated by the restriction endonucleases.

DNA double strand	Restriction endonuclease (RE)	Fragment generated by RE cleavage
-A T G A A T C T G- -T A C T T A G A C-	*Hinf I*	-A T G -T A C T T A
-T C G G C G C T G A- -A G C C G C G A C T-	*Hae II*	-T C G G C G C -A G C
-G G G C C G T T T G G G C C T- -C C C G G C A A A C C C G G A-	*Bgl I*	-G G G C C G T T T -C C C G G C

induced damage(s) than AT-rich sequences. The damage is more likely to be base change or base loss which may alter the restriction site. It is likely possibility that inherent radiosensitivity may be at least partly determined by the GC-richness of NT sequence in the DNA.

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