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FREE-RADICAL YIELDS IN PROTON IRRADIATION OF ORIENTED DNA: RELATIONSHIP TO ENERGY TRANSFER ALONG DNA CHAINS

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ABSTRACT

Spatial patterns of energy deposition on the nanometer scale are currently believed to be a major factor in determining the biological effectiveness of ionizing radiation. If the most common precursors of biologically significant lesions are clusters of ionization in or near DNA, then intramolecular energy and charge transfer along DNA chains could be very important in lesion development. This paper describes investigations of these phenomena through model calculations and measurements of radical yields in oriented DNA exposed to proton irradiation.

INTRODUCTION

The idea that the critical lesions in radiation biology result from clustering of ionizations on a nanometer scale can be traced to the early work of Lea (1947), Howard-Flanders (1958), and Barendsen (1964). More recent support for this model of the biological effects of ionizing radiation comes from the analysis of cell killing by radiations with different linear-energy-transfer (LET) based on computer simulations of track structure in water (Goodhead, 1987). Although these biophysical models ignore the complexity of the cellular medium and the macromolecular structures that regulate its function, their basic conclusion can be rationalized by the high scavenging capacity of the chemical environment of DNA (Roots and Okada, 1975) and the efficiency of repair of minor perturbations of DNA structure (Doetsch and Cunningham, 1990).

From arguments of this type, one concludes that the most common precursor of cytotoxic and mutagenic effects from radiation exposure is a cluster of ionization in or, at least, very near to the DNA molecule. The involvement of macromolecules in the early stages of lesion production opens the possibility of intramolecular energy and charge transfer following excitation or ionization by the radiation field. These processes acting in the presence of traps for energy and charge provide a mechanism for concentrating energy deposited in macromolecular systems that is independent of stochastic processes in the slowing down of charged particles. Conversely, energy and charge transfer along DNA chains may dissipate clusters of excitation and ionization before biologically significant lesions are formed. In general, the existence of energy and charge migration in macromolecules tends to decouple lesion production from the stochastics of energy deposition

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just as ordinary diffusion tends to decouple radiation chemistry on a long time scale from track effects in the radiolysis of homogeneous solutions of small molecules.

Recent findings at several laboratories have raised questions about the assumption that radiation-induced DNA damage remains localized on the nanometer scale during lesion formation. Observations by Arroyo et al. (1986) that the yield of neutron-induced free radicals in oriented DNA fibers was dependent on the orientation of the sample relative to the neutron flux were attributed to energy transfer between stacked DNA bases. Al-Kazwini et al. (1990) presented evidence that electrons can move along DNA chains for distances up to about 100 base pairs. Data obtained by van Lith et al. (1986) on microwave conductivity in pulsed radiolysis suggest that excess electrons in frozen DNA solutions can migrate for distances of the order of 100 nm in the structured water layers around macromolecular chains.

Simultaneous with these experimental results, there has been a renewed interest in nonlinear modes of vibrational excitation in DNA called solitons (Baverstock and Cundall, 1988). Several models for solitary waves in DNA have been proposed (Englander et al., 1980; Yomosa, 1984; Takeno and Homma, 1987; Zhang, 1987, Muto et al., 1988). In Yomosa's model, about 0.4 eV of vibrational energy is transported along DNA at a rate of about 100 nm/ns as a disruption of hydrogen bonds between complementary bases. The broad spectrum of excitation associated with the absorption of energy from ionizing radiation in biological systems (Bednár, 1985) should provide ample opportunity to overcome the energy threshold required to initiate nonlinear phenomena like solitons (van Zandt, 1989).

The first section of this paper discusses models for the effects of energy and charge transfer on free-radical yields in oriented DNA samples exposed to direct proton-beam irradiation. Experiments designed to detect an orientation dependence of radical yields from proton irradiation of oriented DNA are described in the second section. Our conclusions are presented in the final section.

MODELING RADICAL YIELDS IN PROTON IRRADIATION OF ORIENTED DNA

We have investigated (Miller et al., 1988; Miller and Swenberg, 1990) mechanisms for the observation by Arroyo et al. (1986) that DNA base radicals induced by neutrons are dependent on the orientation of DNA fibers relative to the neutron flux. Monte Carlo codes developed by Wilson and Paretzke (1981) and scoring algorithms contributed by Charlton (1985) were used to model energy deposition in oriented DNA under direct proton-beam irradiation. Table I summarizes our results for a 1 MeV proton flux incident on an oriented DNA sample either parallel or perpendicular to the fiber direction. Although the average amount of energy deposited in the parallel case is 5 times greater than in the perpendicular case, the average separation between the excitations or ionizations in the same DNA chain is about 100 times greater in the parallel case. The pattern of deposition in the parallel case is also more sensitive to uncertainty in the fiber orientation relative to the proton beam.

If the pattern of energy deposition events along a DNA chain in the parallel case is as diffuse as these model calculations suggests, then only long-range modes of intramolecular energy or charge transfer could couple the excitations and ionization in the molecule in ways

Table 1. Energy Deposition in Oriented DNA by 1 MeV Protons

Orientation	Energy Deposited (eV)	Event Separation (nm)
0°	293	224
0° ± 10°	150	47
90°	62	2
90° ± 10°	62	2

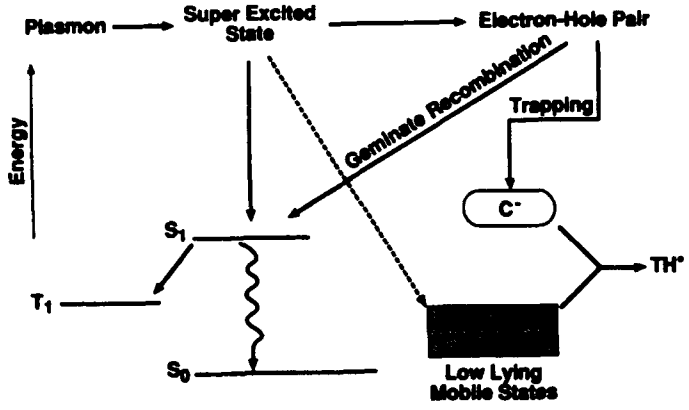

Figure 1. Decay Modes of Energy Absorbed in Solid DNA Samples

that would make the yield of free radicals orientation dependent. For example, recent work by Georghiou (1990) indicates that singlet excitations of bases in calf thymus DNA at room temperature move only 1 or 2 base pairs before they are irreversible trapped. Clearly, this type of excitation has a low probability of interacting with other energy deposition events in same DNA chain and should have equivalent effects in both irradiation geometries.

Energy absorbed from ionizing radiation in oriented DNA fibers should produce modes of excitation that are considerably more mobile than singlet excitons (Al-Kazwini et al., 1990; van Lith et al., 1986; Yomosa, 1984). Figure 1 illustrates a mechanism by which solitons might influence free-radical yields when DNA at 77° K is exposed to a proton beam parallel to the molecular orientation. The interaction of DNA with protons and secondary electrons produces highly excited electronic states that decay primarily through formation of electron-hole pairs. Usually this decay is accompanied by some conversion of electronic to vibrational energy. Sufficiently large vibrational excitations in DNA can be self cohering (van Zandt, 1989). Recent work by Bernhard (1989) suggests that excess electrons are trapped on cytosine by reversible proton transfer from guanine. If many super-excited states are produced in the same DNA chain by a proton flux that is parallel to the molecular orientation, then the probability of interaction between trapped electrons and solitons increases. This interaction may induce electron transfer to thymine where TH· is formed by irreversible protonation at C6.

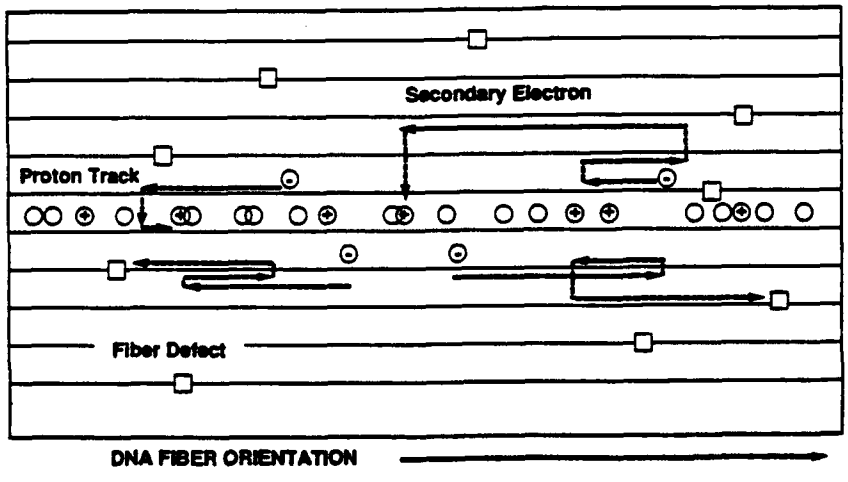

Figure 2. Schematic of Electron-hole Recombination in Oriented DNA Exposed to a Proton Flux that is Parallel to the DNA Fibers.

The model illustrated in figure 1 neglects the mobility of electrons ejected in the decay of super-excited states by ion-pair formation. The high mobility and long lifetime of excess electrons in the hydration layers of DNA (van Lith et al., 1986) could also contribute to orientation effects in DNA damage by proton irradiation. This mechanism is illustrated in figure 2, where the dashed lines represent trajectories of ejected electrons that move primarily in hydration layers around DNA but occasionally are scattered between DNA chains. The squares represent fiber defects that mainly determine the mean free path of excess electrons when the sample is exposed to low LET radiation or protons perpendicular to the fiber orientation. However, when proton tracks are parallel to the fiber direction, many positive ions lie in the high-mobility path of excess electrons. This increases the probability of electron-hole recombination and could reduce the yield of primary radical anions and cation in the parallel case.

EXPERIMENTS WITH PROTON IRRADIATION OF ORIENTED DNA

Preparation of oriented DNA samples (Rupprecht, 1966) to look for effects like those illustrated in figures 1 and 2 involves wet spinning of high molecular weight DNA into fibers that are wound on a spool to form a thin sheet of oriented DNA. Samples for perpendicular

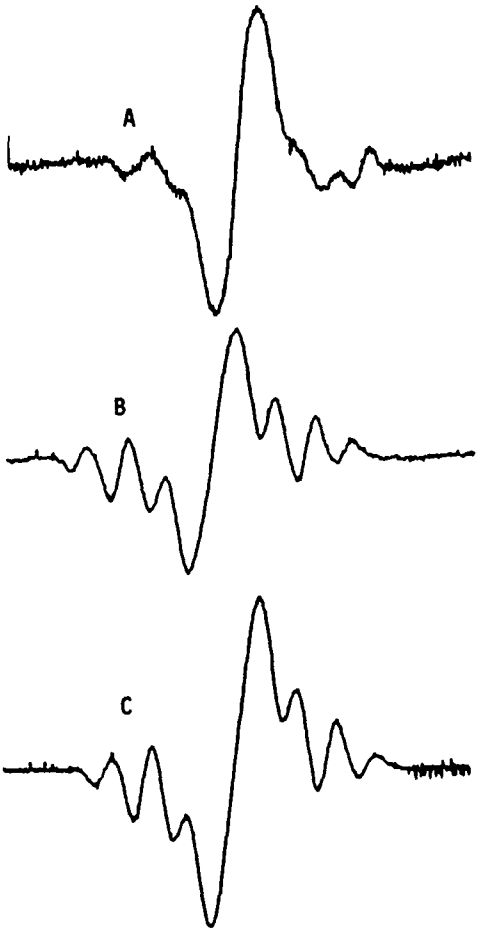

Figure 3. EPR Spectra of Oriented DNA Exposed to γ-rays (A) and Protons Perpendicular (B) or Parallel (C) to the DNA Fibers.

irradiation were made by pressing together a sufficient number of sheets to give a thickness greater than the range of a 4 MeV proton, which is about 0.5 mm. Samples for parallel irradiation were sliced from a block of oriented DNA that had a thickness slightly less than the 3 mm inside diameter of quartz tubes used to transfer irradiated samples to the electron paramagnetic resonance (EPR) spectrometer. All samples were approximately 1 cm long and weighed about 15 mg.

For irradiation, the samples were placed on a copper block in contact with a reservoir of liquid nitrogen and held in place by a thin polyester film. After cooling to 77° K, the sample was placed in a vacuum chamber attached to the beam line of the accelerator. Samples were exposed to graded doses of 4 MeV protons in the range of 20 to 60 kGy. The dose rate of 2.5 kGy/min was less than the value of 10 kGy/min recommended by Henriksen and Snipes (1970) to avoid sample heating. After irradiation, the samples were removed from the vacuum chamber and transferred to a precooled EPR tube. During this transfer, the sample lost contact with liquid-nitrogen cooled surfaces for less than one second as it fell through a funnel into the EPR tube. Several samples were exposed to γ-rays for comparison with published data (Gräslund et al., 1971) and the results of proton irradiation. In this case the sample could be sealed into an EPR tube before irradiation due to the penetrating power of the radiation.

The EPR spectra shown in figures 3A-3C were observed after 4 kGy of γ-rays, 56 kGy of protons incident perpendicular to the fiber orientation, and 48 kGy of protons parallel to the DNA chains, respectively. All three spectra are composed of a central line that we associate with primary radical anion and cation species with varying amounts of modulation in the wings resulting from TH· production. The greater amount of TH· observed with proton irradiation is not likely to be due to sample warming during the transfer from the proton beam line to the EPR spectrometer because the irradiated samples were kept in contact with liquid nitrogen cooled surfaces and the magnitude of the central line relative to the structure in the wings did not change as a function of proton dose. The greater proportion of TH· with proton irradiation may be due to the higher dose required to detect radicals since the total radical yield per unit of dose was more than an order of magnitude lower for protons than for γ-rays; however, the shape of the EPR spectrum did not change significantly with proton dose in the range investigated. The recommendation of Henriksen and Snipes (1970) regarding the dose rate was based on their experience with 6.5 MeV electrons which may not apply to proton irradiation; hence, the higher yield of TH· that we observed with protons may have resulted from sample heating due to a dose rate that was too large.

Unlike the results reported for neutrons (Arroyo et al., 1986), EPR spectra of radicals produced by direct proton irradiation of oriented DNA in parallel and perpendicular geometries were not significantly different. Figure 4 shows that, within experimental error, total radical yields were also independent of the orientation of DNA fibers relative to the proton flux. To obtain these results, differential EPR spectra were recorded digitally and

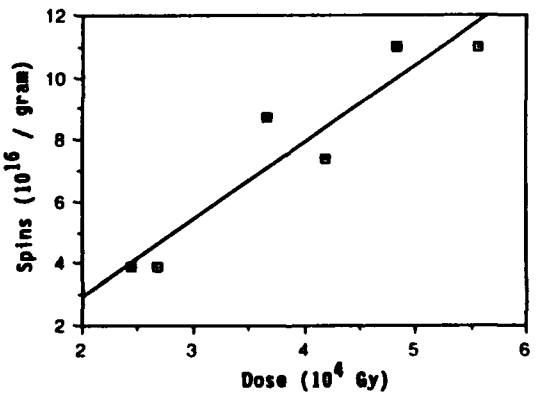

Figure 4. Total Radical Yields in Parallel (●) and Perpendicular (□) Proton Irradiation of Oriented DNA at 77° K.

double integrated to give the area under the absorption lines. This area was converted to number of spins by comparison with a standard sample of 2,2 diphenyl-1-picrylhydrazyl (DPPH) dissolved in paraffin.

CONCLUSIONS

We did not find any evidence for long-range energy or charge transfer in DNA from experiments in which oriented DNA was exposed to direct proton-beam irradiation. This may be due to the high doses and dose rates used in our experiments. The dose and dose rate could be reduced by redesigning the sample holder and transfer system to avoid the limitations on sample size imposed by the present system. Experiments with larger samples, higher proton energies for greater penetration, and improved EPR detection sensitivity might reveal orientation effects that are not present in our data due to sample heating or other processes that destroy free radicals at high exposure levels (Bernhard, 1981).

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