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RADIATION-INDUCED ELECTRON MIGRATION ALONG DNA

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Alfred F. Fuciarelli, Ellen. C. Sisk, John. H. Miller, and John. D. Zimbrick Radiation-Induced Electron Migration Along DNA.

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ABSTRACT

Radiation-induced electron migration along DNA is a mechanism by which randomly produced stochastic energy deposition events can lead to nonrandom types of damage along DNA manifested distal to the sites of the initial energy deposition. Electron migration along DNA is significantly influenced by the DNA base sequence and DNA conformation. Migration along 7 base pairs in oligonucleotides containing guanine bases was observed for oligonucleotides irradiated in solution which compares to average migration distances of 6 to 10 bases for *Escherichia coli* DNA irradiated in solution and 5.5 base pairs for *Escherichia coli* DNA irradiated in cells. Evidence also suggests that electron migration can occur preferentially in the 5' to 3' direction along DNA. Our continued efforts will provide information regarding the contribution of electron transfer along DNA to formation of locally multiply damaged sites created in DNA by exposure to ionizing radiation.

Electrons in Radiation-Induced DNA Damage

Absorption of energy following exposure of aqueous solutions to ionizing radiation initiates a cascade of events involving ionization and energy exchange between excess electrons and molecules within the solvent. Absorption of ionizing radiation by the DNA-water complex results in formation of excited states of DNA and charge separation within the complex. Charge recombination occurs in competition with complete charge separation and electrons which escape recombination processes can migrate along DNA bases (O'Neill and Fielden 1993). Both solvated and unsolvated electrons can be captured by purine and pyrimidine bases in DNA and these electrons can subsequently "tunnel" along DNA in the overlapping pi-electron system created by the stacked bases. Migration of electrons along DNA is an important, but not well understood, mechanism underlying the distribution of radiation damage.

In solid state DNA systems migration of electrons over distances exceeding 25 base pairs has been observed using several spectroscopic methods (reviewed by O'Neill and Fielden 1993). However, short range migration distances on the order of 1-3 base pairs (Yan *et al.* 1992) and 2-6 base pairs (Spalletta and Bernhard 1992) have recently been measured using ESR on frozen preparations of hydrated DNA samples. Assessing radiation-induced electron migration along DNA in aqueous solution has been a more difficult experimental task, however, several studies reveal that electron migration distances in aqueous solutions of DNA (Anderson *et al.* 1991; Fuciarelli *et al.* 1994) and in irradiated cells (Beach *et al.* 1994) are generally less than 10 base pairs.

5-Bromouracil as an Indicator of Electron Interactions in DNA

Our approach for investigating ionizing radiation-induced electron migration in aqueous samples of DNA, involves use of 5-bromouracil (5-BrU), which can be incorporated via a phosphoramidite derivative into synthetic DNA fragments (Fuciarelli *et al.* 1994) or by thymine-substitution in growth media (Beach *et al.* 1994). In aqueous solution, interaction of 5-BrU with solvated electrons results in release of bromide ions

and formation of a highly reactive uracil-5-yl radical capable of capturing hydrogen atoms from substrates in the irradiated solution (Zimbrick *et al.* 1969). In irradiated solutions of 5-BrU, release of bromide ion and formation of uracil occur in a quantitative manner; that is, within experimental error all solvated electrons formed during water radiolysis yield bromide ions and uracil (Zimbrick *et al.* 1969). In a series of experiments in which we used deuterated water and deuterated t-butanol (t-butanol is used to scavenge hydroxyl radicals formed during water radiolysis), we demonstrated that hydrogen atom donation can occur from different sources depending upon whether 5-BrU is irradiated as a monomer in solution or as another base incorporated into DNA (Fuciarelli *et al.* 1994b). When irradiated as a monomer, 5-BrU captures a hydrogen atom from t-butanol. This was observed by irradiating solutions of 5-BrU containing deuterated t-butanol and using gas chromatography-mass spectrometry (GC/MS) methods to demonstrate the presence of deuterated uracil derivative with a molecular mass 1 a.m.u. greater than that observed for uracil due to the presence of a deuteron. However, when 5-BrU-containing oligonucleotides are irradiated in their double-stranded forms, the hydrogen atom is captured from the DNA molecule because irradiated solutions containing deuterated t-butanol resulted in low levels of deuterated uracil (Fuciarelli *et al.* 1994b). This latter observation is consistent with the hypothesis that hydrogen atom donation may occur from the 2' carbon of the 5' adjacent deoxynucleoside in DNA rather than from other hydrogen atom donators in solution (Zimbrick *et al.* 1969; Hutchinson 1973). Molecular models of the orientation of the bromine atom on the 5-BrU moiety in DNA and the hydrogen atom on the 2' carbon of the 5'-adjacent deoxyribose indicate that these atoms are very close to each other in DNA, thereby facilitating hydrogen atom donation (Hutchinson 1973).

Effects of DNA Base Sequence on Electron Migration

DNA base sequence and conformation are major determinants influencing the maximum distance over which electrons can migrate along DNA, and reactions involving

mixtures of DNA bases with solvated electrons provide some preliminary insights in this regard (Nese *et al.* 1992). Additionally, in native DNA, it was hypothesized that the transfer of an electron along the helix axis competes with proton transfer processes including both intra-base pair transfer occurring in the direction perpendicular to the helix axis and extra-base pair transfer from bulk water (Steenken 1992). To examine the effects of base sequence and DNA conformation on electron migration, a set of oligonucleotides containing 5-BrU at selected positions with three base (guanine, cytosine, thymine, or adenine) spacers (e.g., $[BrU(GGG)_3]_3$) were exposed to ionizing radiation in their single-stranded form, or alternatively, in their double-stranded form following annealing with appropriate complementary sequences. Differences in uracil yields, as measured by GC/MS, suggested that electron migration occurred to different extents, in oligonucleotides containing different base sequences. In irradiated single-stranded oligonucleotides, the yield of uracil decreased in the following order: A>T>>C≈G and in irradiated double-stranded oligonucleotides, the yield of uracil decreased in the following order: G>C≈T>A (Fuciarelli *et al.* 1994a). The mechanisms underlying these results may be due to electron transfer-induced changes in the acidity/basicity which leads to corresponding changes in the protonation and charge state of the molecules (Steenken 1992). In addition to differences in the chemical reactivity of a molecule (or radical) for its various protonation states, the source of the proton is also an important consideration. For example, in aqueous solutions of nucleobases, the proton exchange partner is bulk water. However, in DNA, extra-pair hydrogen bonds between the O and N heteroatoms are involved with water molecules in the hydration shell of the DNA and, in the case of double-stranded segments of DNA, the complementary base in the opposite strand can be an important source for intra-pair proton transfer reactions (Steenken 1992). Electron migration along DNA can therefore be influenced by competing proton transfer reactions occurring within DNA base pairs and between DNA and bulk solvent.

Migration Distances Along DNA in Irradiated Solutions

The average distance over which electrons would migrate was determined using a series of oligonucleotides containing 5-BrU at selected positions with guanine spacers (i.e., $[BrU(G)_n]_3$, where n=3, 5, 7, 9). Double-stranded oligonucleotides containing guanine stretches adjacent to 5-BrU moieties were used since our sequence data indicated that radiation-induced uracil yields were greatest under these conditions (Fuciarelli *et al.* 1994a). GC/MS analysis revealed that a significantly lower amount of 5-BrU destruction was evident for oligonucleotides with 9 base spacers compared to oligonucleotides having 3, 5 or 7 base spacers (Fuciarelli *et al.* 1994a). This observation suggested that the average distance for migration does not extend beyond 3 to 4 bases assuming that migration occurs as efficiently in either direction along the DNA molecule. The migration distance could increase to 7 bases if migration proceeds in only one direction and this point will be revisited below.

In another series of experiments, different amounts of 5-BrU were substituted for thymine in medium used to grow *Escherichia coli* cells, and average electron migration distances were assessed in *Escherichia coli* DNA extracted and irradiated in solution, or irradiated in cells (Beach *et al.* 1994). Bromide ion release was assayed using x-ray fluorescence spectrometry (which actually measures bromine atoms) following irradiation. By varying the amount of 5-BrU in the medium, hence the amount incorporated into the DNA, the average distance between 5-BrU molecules was systematically changed and, because the number of 5-BrU/electron reactions was monitored by the amount of bromine released, the maximum average electron migration distance along the 5-BrU-DNA could be estimated. Using this approach, the maximum average electron migration distance in aqueous solutions of 5-BrU-substituted DNA was 6.5 to 10 base pairs (assuming only intrastrand migration). Similar methods revealed charge migration in 5-BrU-substituted DNA in irradiated *Escherichia coli* cells and the maximum average migration distance was 5 to 6 base pairs (assuming only intrastrand migration) (Beach *et al.* 1994).

Preferential Migration of Electrons in the 5' to 3' Direction Along DNA

Analysis of the extent of radiolytic destruction of 5-BrU revealed that electron migration occurred efficiently over a distance of 3 to 4 guanine bases assuming that migration could occur as efficiently in the 5' to 3' direction as in the 3' to 5' direction. The migration distance could increase to 7 bases if migration proceeded preferentially in only one direction. To determine whether electron migration could occur preferentially in one direction along DNA, we synthesized oligonucleotides in which electrons were permitted to move only in one direction. Cytosine acts as an electron sink (Fuciarelli *et al.* 1994c), therefore, electrons would be unable to migrate past them. Oligonucleotides of the following sequence were synthesized: 5'-CCC$(G)_7$BrUC-3' and 5'-CBrU$(G)_7$CCC-3', which permit electrons to migrate only in the 5' to 3', or 3' to 5' direction, respectively. Appropriate complimentary oligonucleotides were synthesized, annealed to create double-stranded DNA, and were used for irradiations. Subsequent GC/MS analysis for uracil formation revealed a significant radiation chemical yield of uracil in 5'-CCC$(G)_7$BrUC-3', but very little uracil was formed in 5'-CBrU$(G)_7$CCC-3' (Fuciarelli *et al.* 1994c). Greater uracil yields in 5'-CCC$(G)_7$BrUC-3' suggested that electrons are capable of preferential migration along DNA containing a segment of guanine bases in the 5' to 3' direction (Fuciarelli *et al.* 1994c). Computer simulation of the relationship between the guanine base immediately adjacent to 5-BrU and the 5-BrU molecule indicates that this preferential migration occurs as a result of greater overlap of the pi-electron clouds of the DNA bases.

Consequences of Electron Migration as a Component of Radiation-Induced DNA Damage

Electron migration is an important process underlying the distribution of radiation damage in DNA, and could help to explain how a nonrandom distribution of DNA damage occurs following energy deposition by stochastic processes. The contribution of solvated electrons and unsolvated electrons that escape recombination processes to free radical-induced damage to DNA represents a uniquely different mechanism leading to

oxidative DNA damage than that of other physical agents such as ultrasonic cavitation (Fuciarelli *et al.* 1993d) or chemical agents such as hydrogen peroxide (Blakely *et al.* 1990). Additionally, the distribution of DNA damage is considerably different with these agents; ionizing radiation creates locally multiply damaged sites and hydrogen peroxide exposure leads to formation of singly damaged sites along the DNA. In cells, singly damaged sites on DNA would be much easier to repair by enzymatic processes than multiply damaged sites, such as those generated by exposure to ionizing radiation. Multiply damage sites in DNA demand a significantly more complex form of enzymatic processing for repair. Increased radiosensitivity of cells containing 5-BrU-substituted DNA (Kinsella *et al.* 1984) could be a consequence of electron migration along the DNA. Increased radiosensitivity potentially could result from increased production of double-strand breaks as a result of migration of radiation damage along one strand of DNA to a position located opposite a single-strand break in the complementary strand within a locally multiply damaged site. However, alternative mechanisms leading to production of a single-strand break on the opposite strand to that containing 5-BrU, including formation of an alkali-labile lesion by bromine, abstraction of a hydrogen bond on the opposite strand by bromine, or a radical transfer reaction involving the reactive uracilyl radical, cannot be discounted.

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