Distribution of Strand Breaks Produced by Auger Electrons in Decay of ^{125}I in Triplex DNA

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Acta Oncologica Vol. 39, No. 6, pp. 707–712, 2000

In this study we investigate the possibility of using Auger electrons as a probing agent for the study of structures of nucleic acids. To this end, we present the distribution of breaks produced in strands of a DNA duplex and a triplex-forming oligonucleotide (TFO) carrying Auger emitting radionuclide ^{125}I. The method of calculation includes use of a molecular model of plasmid DNA duplex with bound TFO carrying a labelled ^{125}I at position C5 of a single deoxycytosine residue, a source of Auger spectra, Monte Carlo electron track structure and the ensuing chemistry codes, to simulate the distribution of breaks produced in both strands of a plasmid DNA. Frequencies of fragment length distributions were obtained for the TFO, the purine and the pyrimidine strands. The frequency of breaks in the purine strand showed good correlation with the published experimental results, while that for the pyrimidine strand is lower by a factor of 3. It is concluded that the true structure of triplex DNA may not be purely of B-form.

Received 10 August 1999
Accepted 15 March 2000

Recently, Panyutin & Neumann (1) carried out an experiment measuring the frequency of DNA damage using a triplex forming oligonucleotide (TFO), labelled with ^{125}I, complementary to the polypurine-polypyrimidine region of the *nef* gene of the human immunodeficiency virus. The experiment was the first attempt to use the labelling of a TFO to deliver radionucleotides to target DNA and to produce sequence-specific DNA breaks. In this report we present modelling and calculations of DNA damage by Auger electrons emitted from an ^{125}I-bearing triplex-forming oligonucleotide bound to the plasmid DNA sequence used in the above experiment.

Auger emitting radionuclides such as ^{125}I, ^{123}I, ^{51}Cr, ^{67}Ga, and ^{111}In are widely used in diverse fields such as Auger electron spectroscopy, nuclear medicine and radiation biology. The unique properties of Auger emitters are due to the dense cascade of electrons released after radionuclide decay by electron capture and/or internal conversion. Most of these electrons are short-range particles with energies of less than 1 keV. Release of these electrons results in a highly charged residual atom and deposition of large amounts of energy near the decay site. Early work on the biological effects of the Auger emitter ^{125}I demonstrated that about one DNA double strand break was induced for each ^{125}I decay in DNA (2) and damage by Auger electrons resembled the characteristics of high LET radiations (3). In another pioneering experiment (4) measurements were made of the distribution of the greatest distances of single strand breaks from the base carrying the iodine in the strand containing the decay, and it was found that most single strand breaks were produced within fewer than 10 base pairs. Other studies showed that the extreme toxicity of Auger emitters may prove valuable not only for basic scientific work, but also for medical use in tumour therapy and diagnostics (5, 6).

In the past two decades substantial efforts have been made to utilize the promising potential afforded by Auger emitting radionuclides in radiation biology for both diagnostic and therapeutic purposes (5, 6). However, use of Auger emitting radionuclides presents major problems in dosimetry, in cancer therapy and diagnostic work. In dosimetry, the calculation of dose is problematic, as electronic equilibrium is not achieved at molecular size targets (7). In diagnostic work, problems with the site specificity have hampered progress in the application of Auger emitting radionuclides. A recent review by a task group for the

 ISSN 0284-186X

American Association of Physicists made a critical review of dosimetry of Auger electrons and provides average Auger spectra of several radionuclides (8, 9).

Modelling and calculation of damage by Auger electrons in more complex molecules have been carried out by a number of workers, for example in nucleosomal DNA (10, 11). There is still an ongoing debate on the level of contributions of OH radicals to DNA damage. Most theoretical calculations indicate a greater share of damage arising from direct energy depositions. In contrast to the well-established data for plasmids, recent experiments carried out by Kassis, Walicka and their colleagues (12, 13) indicate a greater contribution to total DNA damage from OH radicals. Therefore, conformation of DNA in the form of plasmid or higher orders in the cell may have a pronounced influence on the radiotoxicity of the Auger emitting radionuclides.

In this report we present data on the modelling and calculation of DNA damage induced by Auger electrons emitted from radionuclide ^{125}I labelled to a TFO. Data are presented on the distribution of breaks in both strands of the DNA duplex and the TFO strand and are compared with the experimental results.

METHOD

A simplified representation of the triplex DNA used in the experiment conducted by Panyutin & Neumann (1) is shown in Fig. 1. The diagram shows the sequence of the 53 nucleotide base pairs of the plasmid, position of the iodine on the triplex-forming strand (marked as I), the purine and the pyrimidine strands.

The conceptual method of scoring DNA strand breaks induced by decay of iodine is shown in Fig. 2. For illustration purposes, this particular decay of iodine contains 22 electrons of various energies. Interaction of electrons released in the decay of ^{125}I causes direct damage in the DNA molecule and generates radical species in the bulk water around the DNA.

The method of calculation, in principle, follows our previous reports on the calculation of DNA damage by Auger electrons (14, 15). The method accounts for damage by ionisations and excitations in DNA and the diffusing hydroxyl radicals generated in the bulk water surrounding the DNA. It is assumed that no radical is generated in the primary hydration shell (16) and any charge resulting from molecular interaction of electrons with this water is transferred to the polar atom on the back bone or the bases of DNA.

Electron tracks emitted at each radionuclide decay and the distribution of radicals produced were generated using the Monte Carlo track structure code CPA100 (17). The code follows the history of the primary and secondary electrons, interaction by interaction, in liquid water, until they are thermalized and generation of stable radicals species such as OH, H and e_{aq} at 10^{-12} s. In the prechemical stage (10^{-15} s to 10^{-12} s) the ionized and excited water molecules undergo several reactions leading to the formation of free radical species and molecular products. Subexcitation electrons, electrons with kinetic energy less than the first excitation potential (7.4 eV), may recombine with the parent ion according to the Onsager-Debye theory, or thermalize, losing their energy by successive scattering in the medium until they are in equilibrium with the surrounding molecules, leading to the formation of hydrated electrons.

The general outline of the method is first to place the radionuclide ^{125}I at position C5 of the TFO, then to follow all the electrons emitted in each decay until thermalized and note the positions of interactions with the oligonucleotide. Subsequently, the radicals generated in the cylindrical water shell surrounding the triplex DNA were diffused randomly (in 3-D) and reacted from 10^{-12} s to 10^{-9} s and all the reactions and products were recorded. In this calculation, it is assumed that a DNA strand break is entirely due to damage in the sugar-phosphate moiety and that base modifications do not lead to strand break (18, 19). Of all radical reactions with DNA only those of OH were considered to have a probability of inducing strand breaks. For the direct energy deposition, a threshold value of 17.5 eV was assumed for the induction of a single strand

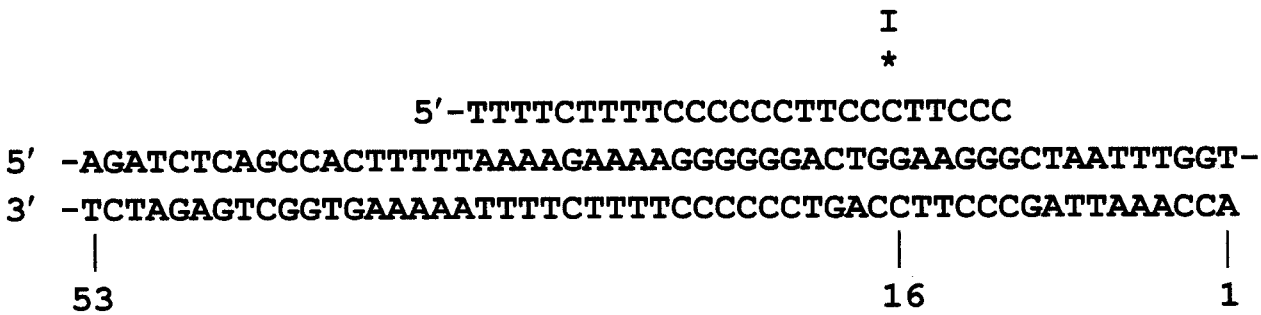

Fig. 1. A simplified representation of the oligonucleotide used in the experiment by Panyutin & Neumann (1). The diagram from top to bottom shows the position of the iodine atom attached to C5 position of the triplex forming oligonucleotide (TFO), the purine and the pyrimidine strands of the plasmid DNA and their sequences.

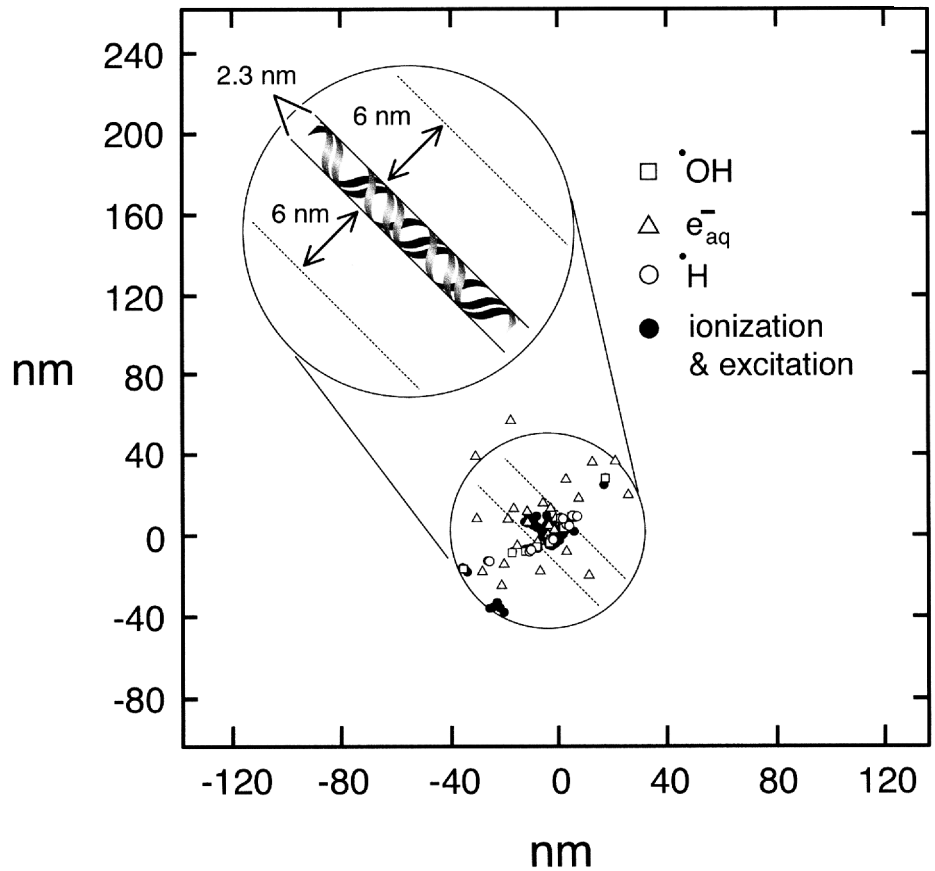

Fig. 2. Diagram illustrating the conceptual method of scoring DNA strand breaks induced by decay of ^{125}I atom. Tracks of all electrons released in 1 decay of the iodine with various energies are projected on the plane of the paper. In this particular case, the decay contained 22 electrons of energies: 86, 422, 39, 65, 63, 15, 34, 34, 3586, 2840, 146, 290, 493, 79, 65, 65, 24, 34, 25, 25, 15 and 15 eV. The inset shows the shaded area—a cylindrical shell around the DNA structure with a variable radius. The radius of the cylinder mimics the experimental condition of the DNA environment. Interaction of electrons released causes ionization and excitation and hydrolysis of the bulk water. The reactive species OH, H and e_{aq} diffuse randomly and could induce DNA damage. Only those reactive species within the 6 nm radius cylinder were diffused. All others were assumed to have very low probability of reaching DNA in a cellular environment.

break (SSB). This value is based on modelling of fragment length distributions produced after decays of ^{125}I in plasmid DNA (14, 20, 21).

The probability of OH radicals causing strand break in the cellular environment was set to 0.13 (22), as not all sugar radicals lead to strand breaks (18, 23). This number is based on the experimental interpretation of damage distribution between the sugar-phosphate and the nucleobases of about 20 : 80 (22, 24). Recent experimental work by Milligan and colleagues (1993) set the efficiency of strand break formation per OH radical attack on sugar-phosphate at about 0.12. It is assumed that hydrated electrons and hydrogen radicals react mainly with the bases to produce base radicals (18, 19). Additional mechanisms such as charge and energy transfer within the DNA spine or the collective excitation were not considered.

All positions of ionizations and excitations were tested for interaction with each individual atom of the triplex structure. This was done by examining whether an event was within the reaction radius of the atom. If the total energy deposited in the reactive volume of the sugar and the phosphate was at least 17.5 eV, this was taken to lead to a single strand break. All radicals generated in the bulk water surrounding DNA within a cylindrical radius of 6 nm were diffused and tested for reaction with the DNA. The coefficients of diffusion and the maximum radius of interaction for OH, e_{aq} and H were set according to previously published data (14, 15, 25).

MODEL OF TRIPLE-HELIX DNA

The molecular model for the plasmid DNA duplex with bound triplex-forming oligonucleotide was based on recent extended molecular dynamics (MD) calculations of DNA triplex structure (26). These have shown that, regardless of initial conformation, MD simulations of triplexes converge to an envelope of conformations that most closely resemble the *B-form* model of the triplex (27) rather than the alternative *A-form* (28) or recent *P-form* (29) models. On the basis of these simulations, a time-averaged model of

the DNA triplex $d(A)^*_{10}d(T)^*_{10}d(T)_{10}$ was produced. Using the molecular graphics package, MidasPlus, the complete duplex/triplex structure was built up from overlapping 10-base fragments of either the above triplex for the central triple-stranded section or of the duplex $d(T)_{10}d(A)_{10}$ in the standard B-form conformation (30) for the double-stranded terminal sections. The fragments were overlapped by least-squares fitting of the terminal base pairs or triples, so that after deletion of duplicate bases the whole structure had a reasonable backbone geometry. Bases were then *mutated* from T or A to G or C as required to generate the correct overall sequence. In Fig. 3 we present the molecular model of the B-DNA duplex and the triplex-forming oligonucleotide.

SIMULATION OF STRAND BREAKS BY THE DECAY OF INCORPORATED ^{125}I

The experiment by Panyutin & Neumann used a plasmid containing 750 bp PCR (polymerase chain reaction) fragment of HIV. DNA samples with ^{125}I were kept at $-20°C$ for 14 and 56 days to accumulate decays. After the accumulation of decays, the TFO was removed and the breaks in the strand, nearest to the TFO (Fig. 1), of the plasmid DNA were counted using sequencing polyacrylamide gel electropheresis (PAGE). The breaks produced by decay of [^{125}I]dC were mapped to the expected positions of the plasmid DNA by comparison with the positions of the bands produced by Maxam-Gilbert sequencing reactions.

In the simulation of the above experiment, the individual electron spectra emitted in the decay of ^{125}I, placed at the same position as in the experiment, were generated and the energy deposition patterns were analysed for induction of single strand breaks.

Efficiencies of single strand breakage in the purine and the pyrimidine strands of the plasmid DNA and in the TFO strand are presented in Fig. 4. The calculated data show good agreement for the frequencies of single strand breaks for the purine strand in comparison with the experimental data. The data show that in the strand nearest to the decaying iodine atom there is a rapid decrease in the average energy deposited away from the location of the decay. This phenomenon can be utilized to deduce information on molecular structures associated with DNA damage in nucleoprotein and nucleic acid complexes.

SUMMARY AND CONCLUSIONS

In this study we presented the frequencies of DNA strand breaks induced by Auger electrons released in the decay of

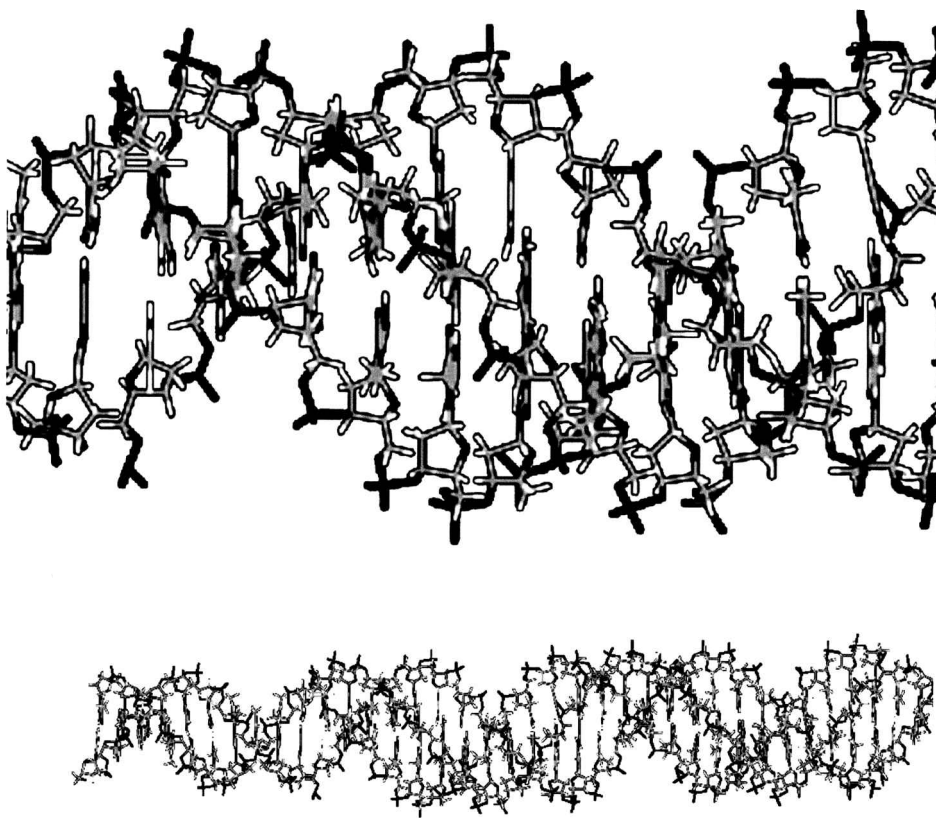

Fig. 3. Three-dimensional structure of the B-form triplex model. The inset shows details of the structure at vicinity of the site of incorporation of iodine. Distances from the iodine to sugars and phosphates have a strong influence on the level of damage to the Pu and the Py strands.

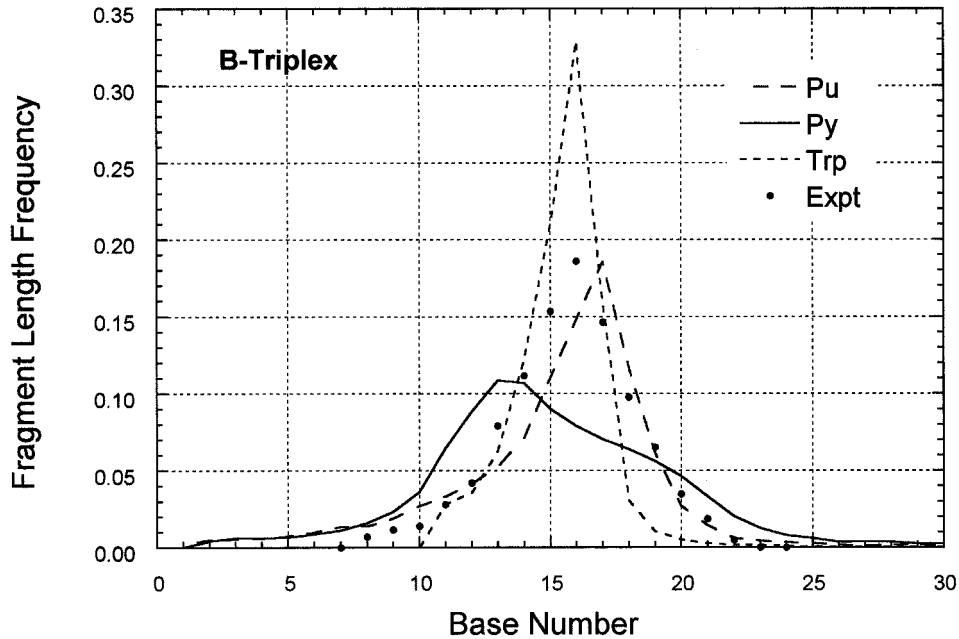

Fig. 4. Distribution of largest fragment lengths produced to the left of the iodine on triplex forming (TFO) strand and both the purine and the pyrimidine strands of the duplex.

^{125}I in a B-triplex DNA. The electron tracks were simulated in liquid water including the radical species generated in the bulk water surrounding the DNA. An energy deposition of 17.5 eV or more, without a priori assumption of the type of interaction (ionization or excitation), in the volume of sugar or phosphate was assumed to lead to induction of a single strand break in DNA. Similarly, a reaction of OH radical with the sugar or the phosphate was given a probability of 0.13 of inducing a strand break. The distribution of breaks in the purine strand of the B-triplex shows good agreement and correlation with the experimental data. The data for the pyrimidine strands do not correlate well with the experimental data. Evidence from the experiment by Panyutin & Neumann indicates similar intensities for both strands of the duplex within the experimental errors. The differences observed in the symmetry of breaks around the site of the decaying radionuclide arise from differences in the triplex structures used in the calculations and the experiment. However, we are not yet certain to what extent conformation of the triplex structure is responsible for the observed differences (data not shown) in frequencies of strand breaks in purine and pyrimidine strands. Although, triple helices have been known to exist for more than four decades, we still do not know whether these structures occur naturally in the cells nor the nature of their DNA structures. We are not yet certain whether the molecular structure of the triplex is of the A-, B-, P- or hybrid form. We have undertaken further investigation to obtain insight into this problem and in conjunction with molecular dynamics and experimental evidence to probe structures of unknown oligonucleotides and associated nucleoproteins using Auger electrons.

ACKNOWLEDGEMENTS

This work was partly supported by the Commission of the European Communities contract no. FIGH-CT-1999-00005. We thank Adrian Ford for help with preparation of the manuscript.

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