Free Rad. Res. Comms., Vols. 12-13, pp. 465-467
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MOLECULAR MECHANISMS OF RADIATION INDUCED DNA DAMAGE: H-ADDITION TO BASES, DIRECT IONIZATION AND DOUBLE STRAND BREAK

R. OSMAN, L. PARDO*, J. BANFELDER, A.P. MAZUREK,
L. SHVARTZMAN, R. STRAUSS and H. WEINSTEIN

Department of Physiology and Biophysics, Mount Sinai School of Medicine of the City University of New York, New York, N.Y. 10029, USA

Structures and properties of G·(-H) and of TH· were obtained from quantum mechanical calculations. New AMBER parameters for these radicals were obtained to fit their structures and charge distributions. Molecular mechanics simulations of the conformational changes induced in a 12-mer of DNA, d(CGCGAATTCGCG), by these radicals show that the distances between the base and the C_2' of the sugar becomes shorter. Such changes suggest that the base radical can abstract the H_2' and transfer the radical from the base to the sugar. Once the radical becomes centered on the sugar a strand break can follow. A simultaneous formation of guanine and thymine radicals on opposite strands may lead to a double strand break.

KEY WORDS:

INTRODUCTION

Approximately 80% of the primary events induced by ionizing radiation are centered on the bases of DNA.[1] This includes indirect effects, where the reactive species (OH and H radicals) formed by ionizing radiaiton in the surrounding environment will add to the double bond of the bases, as well as direct effects, where the energy is deposited directly on the bases of DNA. Guanine is the main target of direct ionization of DNA producing a radical cation, G+ ·, which deprotonates to yield the radical G·(−H). Electron capture by thymine produces a radical anion, T− ·, which was shown to yield the radical TH· upon protonation.[2]

Here we summarize the results of our theoretical study of the mechanism of addition of a hydrogen radical to the C_5-C_6 double bond of thymine, and the computed properties of the products obtained by direct ionization. Because these radiation lesions of the bases cannot directly induce a strand break, we explore the hypothesis that the break results from the transmission of the radical damage centered on the bases to the sugar, through a mechanism of H abstraction by the base.

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*Permanent address: Department of Biostatistics, Faculty of Medicine, Universidad Autonoma de Barcelona, Bellaterra, Barcelona, Spain.

RESULTS

Direct Ionization: The Guanine Radical and Mechanism of Radical Transfer to the Sugars

Quantum mechanical calculations of the neutral guanine radical G·(-H), formed after the loss of the proton on N_1, indicate a planar structure in which the spin is localized on N_3, C_5 and most importantly on C_8. To explore the conformational changes caused in the DNA by the formation of these species we used the molecular mechanics programs in the AMBER package.[3] In order to describe the species formed by radiation damage it was necessary to construct new parameters for the empirical potential used in this package. These parameters were derived from the properties of the species calculated with *ab-initio* quantum mechanical methods. The simulations of the conformational changes due to the radical damage were carried out for a double stranded DNA d(CGCGAATTCGCG) in which G was replaced by the radical G·(−H). Significant change in the hydrogen bonding network between G·(−H) and C are observed due to the repulsion of the lone pairs N· in G·(−H) and N_3 in C. The calculations also show that C_8, which carries part of the spin, is 3.25 Å away from C_2' of the sugar moiety of the previous nucleotide in the same strand (cytosine in our simulation). At this proximity, the guanine radical can abstract the H_2' hydrogen from the sugar, yielding a radical centered on $C_2'$of deoxyribose. After the radical formation on C_2', a β-bond cleavage and elimination of the 3′-phosphate can occur through the same mechanism proposed[4] for β-cleavage from radical on C_4'. This leads to a single strand break of DNA.

Hydrogen Addition to Thymine and Radical Transfer to the Sugar

Radicals add preferentially to the C_5–C_6 double bond of pyrimidine bases[5]. We have selected to investigate the addition of H· to thymine because similar products can be obtained by direct ionization and electron capture by thymine to yield the radical TH·. The structures of the transistion states and of the products differ if the H· addition is to C_5 or to C_6. When H· adds to C_5, the transition state occurs at a distance of 1.84 Å and yields a product in which the ring is puckered at C_5 and the radical is centered on C_6 (TH_5·). When the addition is to C_6 the transistion state occurs at a distance of 1.87 Å and leads to a planar structure with the radical centered on C_5 (TH_6·). The molecular mechanics simulations of a 12-mer of double stranded DNA in which T was replaced by the radical TH· either in position 5 or 6 show that in TH_5· the atom C_6 which carries the radical is at 3.79 Å from C_2' of the sugar moiety of the previous nucleotide in the sequence of the same strand (adenine in our simulation). This distance became 0.14 Å shorter than in regular DNA due to the base puckering which puts C_6 closer to C_2'. From similar simulations with TH_6· the C_5 atom is found 4.20 Å away from C_2', i.e., slightly farther than in regular DNA where it is at a distance of 4.15 Å. We conclude that the base radical TH_5· can abstract the H_2' hydrogen from the sugar to yield a radical centered on C_2' of deoxyribose. It is noteworthy that the atoms proposed to abstract the hydrogen from the sugar are C_6 in T and C_8 in G, which are equivalent in reactivity because both are in α positions to the glycosyl bond. Once the radical beomes localized on the sugar following H abstraction by the base, β-cleavage causes a strand break as discussed above.

Mechanism Of Double Strand Break

By the mechanisms discussed above, the radical can be transferred to the sugar either following ionization of G to produce G·(−H) or following the electron capture by T which yields the radical TH·. If G and T were located on different strands, the β-cleavage process following radical production in both sugars could produce a double strand break and a lethal lesion.

Acknowledgements

This work was supported by the USDOE grant DE-FG02-88ER60675. Generous grants of computer time from the University Computing Center of the City University of New York, the Pittsburgh Supercomputer Center, the Center for Theory and Simulation in Science and Engineering (Cornell University), and the Advanced Scientific Computing Laboratory of the Frederick Cancer Research Facility fo NIH-NCI, are gratefully acknowledged.

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Accepted by Prof. G. Czapski