Primary Oxidation Radical in X-irradiated 5-Methylcytosine, a Mutagenic Hotspot in DNA.

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Recently, methylated CpG sites have been established as mutational hotspots in DNA, one reason of which being oxidation of 5-methylcytosine.[1] Up to 4% of the cytosines in DNA are 5-methylcytosine (m^5C).[2] The ionization potential of m^5C is comparable to that of guanine and m^5C may compete with guanine as a hole trap. Holes that are formed by ionizing radiation in the base regions of DNA may thus subsequently transfer to m^5C.[3] Once oxidized; the m^5C radical may irreversibly deprotonate at the methyl group and form a highly stable radical known as the 3αH radical[4] with a lifetime sufficiently long to impair normal enzymatic activity. To better understand the electronic properties of m^5C as a hole trap, and the mechanisms that form the stable end product, the structure of the initial electron-loss product needs to be determined. Single crystals of 5-methylcytosine hemihydrate and 5-methylcytosine hydrochloride have been X-irradiated at 8-10 K and studied at this and elevated temperatures using EPR, ENDOR, and EIE spectroscopy in addition to single molecule- and cluster DFT calculations at the B3LYP level. The primary oxidation radical is stable only at very low temperatures. Both the experimental results and the DFT calculations indicate a structure where a large part of the electron spin is coupled with the methyl protons. At 10 K the methyl group is not able to rotate classically but undergoes a hindered rotation by tunneling through a rotation barrier at a frequency of ≈100 MHz indicating a barrier of 1-2 kcal/mol. The radical appears to deprotonate at different sites in the two distinct crystalline systems, indicating additional influences of the surroundings. The driving force for deprotonation at various sites has been calculated by DFT. Upon thermal annealing, the primary oxidation radical seems to recombine rather than transform into the 3αH radical, suggesting a high barrier for deprotonation at the methyl group. However, the driving force is greater than for deprotonation at other sites and at room temperature the 3αH radical is the only observed oxidation product in m5C.

1) Chen J.X.; Zheng Y.; West M.; Tang M. Cancer Research 1998, 58, 2070-2075. 2) Erlich, M. Oncogene 2002, 21, 5400-5413. 3) Krivokapić, A; Sagstuen, E. Journal of Physical Chemistry A 2003, 107, 9561-9566. 4) Krivokapić, A; Hole, E. O.; Sagstuen, E. Radiation Research 2003, 160, 340-354.

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