•H Atom and •OH Radical Reactions with 5-Methylcytosine

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The reactions between either a hydrogen atom or a hydroxyl radical and 5-methylcytosine (5-MeCyt) are studied by using the hybrid kinetic energy meta-GGA functional MPW1B95. •H atom and •OH radical addition to positions C5 and C6 of 5-MeCyt, or •OH radical induced H-abstraction from the C5 methyl group, are explored. All systems are optimized in bulk solvent. The data presented show that the barriers to reaction are very low: ca. 7 kcal/mol for the •H atom additions and 1 kcal/mol for the reactions involving the •OH radical. Thermodynamically, the two C6 radical adducts and the •H-abstraction product are the most stable ones. The proton hyperfine coupling constants (HFCC), computed at the IEFPCM/MPW1B95/6-311++G(2d,2p) level, agree well with B3LYP results and available experimental and theoretical data on related thymine and cytosine radicals.

Introduction

Considerable attention has been given during the last three decades to the study of reactions mediated by water radiolysis species including •OH radicals and •H atoms with nucleic acid components.[1−3] In this respect substantial information is now available on the transient radicals produced by the reactions of •OH radicals with the main DNA pyrimidine (thymine and cytosine) and purine (adenine and guanine) bases as inferred from pulse radiolysis, ESR, and theoretical studies.[1,2] In addition, most of the final decomposition products that arise from the •OH-mediated purine and pyrimidine radicals in aerated aqueous solution have been isolated and identified.[3−5]

Altogether the kinetic and structural data thus obtained allow the proposal of comprehensive radical oxidative degradation pathways for the main DNA nucleobases. This provides basic information needed for a better understanding of the molecular effects of endogenously generated •OH radicals and environmental oxidizing agents including ionizing radiation and UVA light on cellular DNA.[6,7] In contrast there is still a relative paucity of information on both the •OH radical- and •H atom-mediated reactions of 5-methylcytosine (5-MeCyt)[8,9] present in DNA as the result of enzymatic methylation of cytosine residues to the extent of a few percent. Despite its low abundance, 5-MeCyt appears to play a major biological role since mutational hotspots have been observed in, for example, the key *p53* tumor suppressor gene at methylated CpG sites.[10] This may be explained, at least in part, by oxidation of 5-MeCyt residues by reactive oxygen species released during aerobic metabolism. Evidence for the occurrence of oxidation reactions involving the methyl group and the 5,6-double bond of the pyrimidine ring of 5-methylcytosine residues in free bases, oligonucleotides, or DNA upon exposure to •OH radical in aerated aqueous solutions has been provided through the isolation of the final degradation products.[11−14]

The formation of 5-formylcytosine and 5-(hydroxymethyl)-cytosine can be rationalized in terms of initial formation of the allylic 5-(cytosyl)methyl radical as a result of •OH radical-mediated hydrogen abstraction from the methyl group. Subsequent fast O_2 addition to the latter radical intermediate gives rise to the related peroxyl radical, the likely precursor of the stable methyl oxidation products of 5-MeCyt either directly as the result of ROO• radical dismutation or indirectly from the decomposition of 5-(hydroperoxymethyl)cytosine, the reduction product of peroxyl radical.[15,16] On the other hand, the formation of 5,6-dihydroxy-5,6-dihydro-5-methylcytosine, the so-called 5-methylcytosine "glycol", is accounted for by initial addition of •OH radicals at C5 and/or C6 followed by the generation through O_2 reaction of the corresponding peroxyl and peroxide precursors.[15,16]

The present theoretical study is aimed at gaining insights into the physicochemical features of the main pyrimidine radicals that arise from the reaction between the •OH radical and 5-MeCyt. These include 6-hydroxy-5,6-dihydro-5-methylcytos-5-yl (6-OH-5-yl) and 5-hydroxy-5,6-dihydro-5-methylcytos-6-yl (5-OH-6-yl). The study was also extended to include the two main pyrimidine radicals that result from •H atom reaction with 5-MeCyt, namely 5,6-dihydro-5-methylcytos-5-yl (6-H-5-yl) and 5,6-dihydro-5-methylcytos-6-yl (5-H-6-yl) radicals, for which there is a lack of information of the type available for the •OH-mediated radicals.

A few theoretical studies have been reported, concerning •H radical addition to the C5 and C6 positions of cytosine[17−19] and the related addition reactions to thymine. Concerning the mechanism for addition of the •OH radical, a preliminary study has been reported by Eriksson.[19] The addition of •OH radicals to thymine was also studied by Jolibois and Morell.[20,21] In these, the energetics and reaction paths were determined. Magnetic properties (hyperfine coupling constants, HFCCs) of the resulting addition radicals are also assessed theoretically herein and compared with the little experimental data available for this family of compounds.[18,20,22,23]

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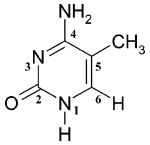

Figure 1. 5-Methylcytosine; schematic drawing including atomic labeling.

Theoretical Method

All systems were investigated by using the hybrid meta-GGA functional MPW1B95[24] as implemented in the Gaussian03 package.[25] The reactants, transition states, and products were optimized at the MPW1B95/6-311G(d,p) level, embedded in the integral equation formalism of the polarized continuum model (IEFPCM).[26−28] Frequency calculations were performed on the optimized structures at the same level of theory, to verify the correctness of the stationary points, and to extract thermal corrections, including zero-point vibrational energies, to the final free energies at $T = 298$ K. Intrinsic reaction coordinate (IRC) calculations were performed on the optimized transition state geometries, to verify that these connected to the given initial reaction complexes and products. Finally, single point energy calculations were performed at the IEFPCM/MPW1B95/6-311++G(2d,2p) level to obtain electronic energies corrected for solvation. To these the free energy corrections specified above were added, for the final free energies reported herein. Throughout, water was used as solvent, as incorporated through the value 78.3 for the dielectric constant.

We herein report total free energies of •H atom and •OH radical addition to C5 and C6 carbons of 5-MeCyt, and for the hydrogen abstraction by the •OH radical from the C5 methyl group. In addition, spin densities as obtained from Mulliken populations and isotropic proton hyperfine coupling constants (HFCC) of the products are discussed. The latter results from the interaction of the unpaired spin distribution with the magnetic nuclei in the system. The suitability of density functional based methods for the evaluation of HFCCs has previously been explored in great detail,[29−32] including •H and •OH radical adducts to cytosine and thymine.[17,18,22,32] The current study is, however, to the best of our knowledge, one of the first applications of the novel kinetic energy corrected hybrid meta-GGAs to radical HFCCs. To verify the correctness of the current approach, B3LYP data were also evaluated on the reaction pathways and for the computed HFCC. As we herein only report proton hyperfine couplings, the current basis set level is highly sufficient, as shown in numerous previous studies.

Results

1. Hydrogen Atom Addition. The atomic labeling of 5-MeCyt is shown in Figure 1, and in parts a and b of Figure 2 we report the optimized structures and main geometric parameters for the pyrimidine reactants, transitions structures, and radical products arising from •H-addition to carbons C5 and C6. In the optimized reactant complexes for addition to both C5 and C6, the hydrogen atom resides far away from the ring (3.5−3.9 Å), and is located right above the C5═C6 double bond. The H−C distances in the transition states are 1.87−1.88 Å, and are associated with quite large imaginary frequencies: 662.1*i* cm^{-1} for 5-H-6-yl radical and 792*i* cm^{-1} for 6-H-5-yl radical. In the H-C5 TS, the hydrogen is positioned straight above C5, whereas in the TS leading to the 6-H-5-yl radical, the hydrogen lies above C6 but slightly outside the ring (H--C6−H angle 83.8°). In the transition states, ca. 0.15−0.2 of the unpaired spin is relocated from the hydrogen onto 5-MeCyt, primarily at the neighboring C6/C5 carbon. In the TS leading to the C5 hydrogen atom adduct, the C5═C6 bond is slightly elongated due to the reduced π-interaction resulting from the formation of the new covalent H−C5 bond (R(C5═C6) = 1.368 Å compared to 1.350 Å in free 5-MeCyt and in the reactant complexes), and we can see an emerging puckering at C5 in that the methyl group carbon is starting to move out of the ring plane. In the H-C6 TS, on the other hand, the structure is essentially unaffected, except for a slight elongation of the C5═C6 bond to 1.369 Å.

The C−H distances in the transition structures obtained with the MPW1B95 functional are slightly shorter than those obtained with the B3LYP functional (1.94−1.95 Å), and also somewhat shorter than those observed at the B3LYP/6-31G(d,p) level for, e.g., addition to thymine (1.98−2.01 Å).[18] The relative difference in activation free energy is similar between the current B3LYP data for 5-MetCyt and earlier reported data for thymine: [18] in the range 0.7−0.9 kcal/mol. At the MPW1B95 level, the difference between the two barriers is larger—approximately 2.4 kcal/mol—in favor of C6 addition.

The 5-H-6-yl radical structure displays considerable puckering of the ring at C5, in agreement with previous observations on radical addition to C5 in cytosine, 1-methylcytosine, and thymine.[22] The unpaired spin is transferred almost entirely to C6, and the C5−C6 bond is now closer to that of a carbon−carbon single bond (R(C5−C6)=1.482 Å). For the 6-H-5-yl radical, on the other hand, the planarity of the ring is essentially maintained, with the two C6-hydrogens positioned above/below the ring plane. The C5−C6 bond is somewhat shorter than that for the C5 adduct, 1.468 Å, and the unpaired spin is more delocalized within the ring although the main fraction (0.64 e) remains at C5.

In Figure 3a we present the computed free energy surfaces for •H-radical addition to C5 and C6 of 5-MeCyt. In the figure, the relative energy differences between the two reactant complexes are also taken into account. In Table 1 we list the corresponding absolute free energies at the IEFPCM/MPW1B95/6-311++G(2d,2p) level, the thermal correction to the free energies, and the relative energies within each reaction. The •H-C6 reaction complex lies ca. 1.3 kcal/mol higher in energy than the H-C5 RC, but the barrier to addition is ca. 2.3 kcal/mol lower once the addition complex is formed. This is in agreement with the findings for •H atom reaction with thymine and cytosine where prereactive compounds were observed.[17,18] The barriers to addition which are relatively low, within the 5−7 kcal/mol range, are indicative of fast reactions. Thermodynamically, the 6-H-5yl product is ca. 10 kcal/mol more stable than the 5-H-6yl one.

The same trends are observed when studying the reaction by using the B3LYP functional (all other parts being the same). As expected, the B3LYP barriers are slightly lower (5.8 kcal/mol for addition to C5, and 4.9 kcal/mol for C6 addition), and the products 1−1.5 kcal/mol less exergonic.

The computed proton HFCCs of the different radical products are listed in Table 2, along with the corresponding experimental electron spin resonance data for 5-MeCyt, thymine, cytosine, and 1-methylcytosine (1-MeCyt), as available. In the 5-H-6-yl radical, the main part of the unpaired spin resides on C6, and we hence observe a large negative coupling on the α-proton, named H6. The hydrogen added to C5 is in an axial position (involving the smallest structural distortion of the large methyl group from the ring plane), which leads to a large hyperconjugative interaction with the π-orbital (the singly occupied molecular orbital, SOMO, on C6) and a very large positive

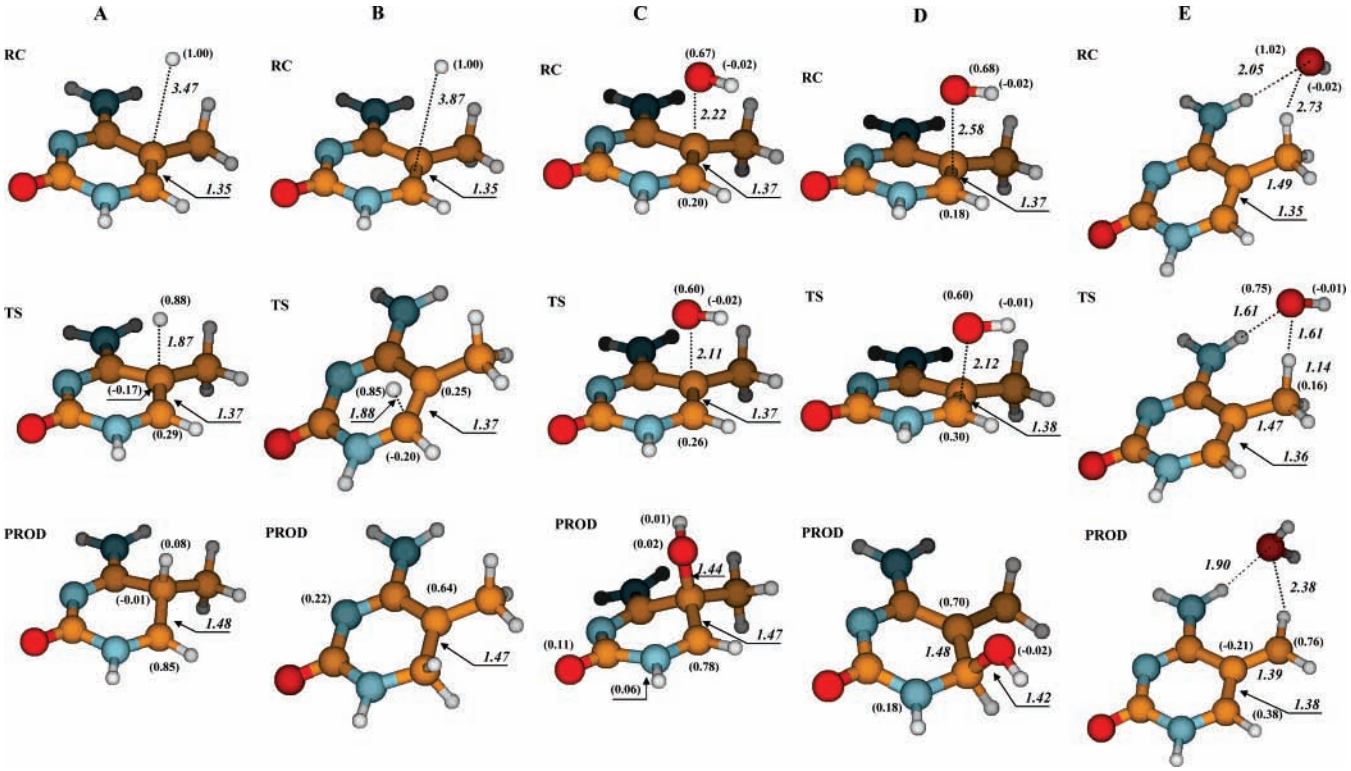

Figure 2. Optimized reactant complexes, transition states, and products for (a) HC5, (b) HC6, (c) OHC5, (d) OHC6, and (e) OH-allyl formation.

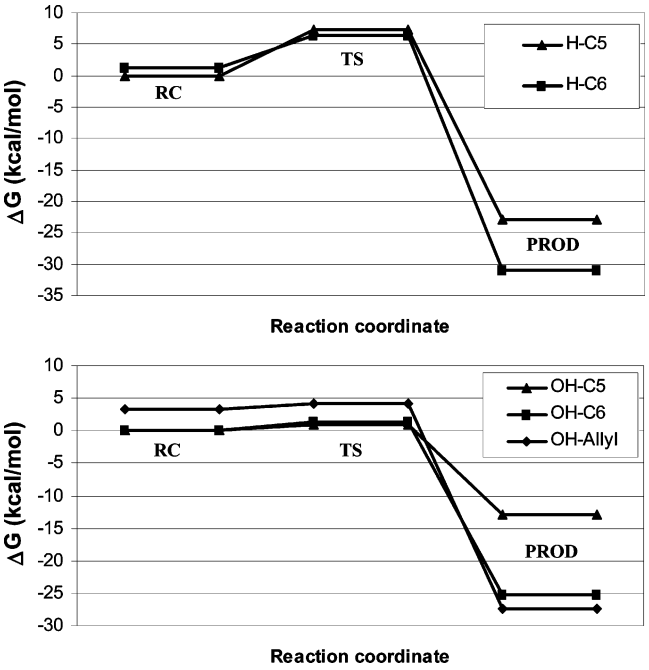

Figure 3. Relative free energies of reaction. Top: H-radical addition to C5 and C6. Bottom: OH radical addition to C5 or C6, or hydrogen abstraction (allyl formation). See text for details.

HFCC. The computed data for the 5-H-6-yl radical of MeCyt correlates very well with available experimental data from cytosine monohydrate crystal studies and thymine single-crystal data.[23,33−35] For the cytosine 5-H-6-yl radical, the puckering of the ring makes the two hydrogens on C5 take axial and equatorial orientations, respectively, resulting in one large and one smaller isotropic HFCC.

In the 6-H-5-yl radical, the ring is essentially planar, and the main components of the unpaired spin are found on C5 and on N3. The SOMO is of π-symmetry, and results in large positive

TABLE 1: Absolute Energies Including Solvation Free Energies (Aqueous Solvent), Thermal Corrections to the Free Energies at $T = 298$ K, and Final Relative Free Energies for the Reactions Studied

system		$E + \Delta G_{aq}$ (au)[a]	ΔG^{298} (au)[b]	$\Delta\Delta G_{aq}^{298}$ (kcal/mol)
H-C5	RC	−434.732095	0.089390	0.0
	TS	−434.726190	0.095191	7.4
	PROD	−434.780304	0.101213	−22.8
H-C6	RC	−434.73214	0.09152	0.0
	TS	−434.726393	0.093774	5.0
	PROD	−434.788617	0.096581	−32.3
OH-C5	RC	−509.982623	0.100384	0.0
	TS	−509.982656	0.101791	0.9
	PROD	−510.006952	0.104145	−12.9
OH-C6	RC	−509.982612	0.100472	0.0
	TS	−509.981846	0.101754	1.3
	PROD	−510.022149	0.099674	−25.3
OH-Allyl	RC	−509.977062	0.100114	0.0
	TS	−509.975731	0.100277	0.9
	PROD	−510.024179	0.098312	−30.7

[a] IEFPCM/MPW1B95/6-311++G(2d,2p) level. [b] IEFPCM/MPW1B95/6-311G(d,p) level.

HFCCs on the two C6-protons and the methyl protons through hyperconjugative interaction. The relative size of the β-HFCCs on C6, and the possible difference between these, depends mainly on the amount of ring puckering induced in the pyrimidine radical, and is often stabilized by the local surrounding. For the methyl group protons, the average in the 6-H-5yl radical of 5-MeCyt is 47.8 MHz, in agreement with the rotational average of 56 MHz seen in the corresponding HC6 radical of thymine.

The data obtained at the current MPW1B95 level of theory are also compared with data for the corresponding products obtained by using the B3LYP functional, and with previously calculated data for H-atom adducts to thymine and cytosine. We note that the static HFCCs obtained at the two different levels agree very well, indicating that MPW1B95 is indeed as

TABLE 2: IEFPCM/MPW1B95/6-311++G(2d,2p) Computed Isotropic Proton HFCCs of the Five Radical Products and Comparative Experimental Data on Single Crystals[23,33−35] or Previous Computational Data.[17,22,a]

system		H1	H(N4)	H(Me)	H6	HX
HC5	calcd	−7.4	0.8/0.2	−1.5/−1.9/−2.2	−45.7	129.5
	exptl, C•H_2O				−52.4	132
	exptl, T				−53.8	136.1
	calcd, B3LYP[b]	−7.6	0.3/0.1	0.2/−1.9/−2.1	−45.0	129.5
	calcd, T[c]				−49.1	113.8
	calcd, C[c]				−52.0	105.9
HC6	calcd	−3.8	−2.4/−3.7	75.9/66.5/1.1	132.4	126.4
	exptl, 1mC•H_2O				143.7	133.7
	exptl, T			3 × 56.0	126.8	89.6
	calcd, B3LYP[b]	−3.4	−2.2/−3.4	74.4/66.5/1.7	129.2	123.1
	calcd, T[c]			3 × 59.9	91.9	91.9
	calcd, C[c]				146.4	146.6
OHC5	calcd	−7.7	−0.9/−1.0	3.9/−1.4/4.0	−51.0	14.1
	calcd, B3LYP[b]	−8.1	−1.1/−1.0	6.8/−1.5/4.8	−49.7	16.0
OHC6	calcd	−0.5	−3.4/−4.9	92.2/76.7/1.5	56.6	6.0
	calcd, B3LYP[b]	0.0	−3.1/−4.8	99.7/63.6/5.5	48.0	7.3
OHAllyl	calcd	−6.4	0.0/0.2	−44.3/−46.8	−25.6	
	exptl, 5mC			−45.8 to −46.5/−46.5 to −47.4	−21.7 to −24.9	
	exptl, T			−44.0/−45.9	−30.0	
	calcd, B3LYP[b]	−6.0	−0.1/0.2	−42.4/−45.0	−25.7	

[a] All data in MHz. HX is the hydrogen added to the ring or present in the hydroxyl radical. [b] Current work on 5mC radicals, using B3LYP functional. [c] B3LYP data including vibrational averaging and thermal effects ($T = 77$ K).

accurate in predicting radical hyperfine properties as the latter, more commonly employed, functional. We also note, based on comparison with previously computed data for the corresponding thymyl or cytosyl radicals,[17,22] that vibrational averaging and thermal effects also can play a role in adjusting the static data reported herein.

2. •OH Radical Attack. The hydroxyl radical is in general considered to be the most reactive oxygen species alongside singlet oxygen, although the latter exhibits a much more selective reactivity, with guanine being the only one DNA target.[36] The addition reactions of •OH are associated with very low barriers, and essentially unselective reactivity. This holds true also in the present study, in that the barriers to addition at C5 and C6 are very similar, ca. 1 kcal/mol, as is also the barrier to hydrogen abstraction at the C5 methyl group giving an allylic-like radical; cf. Table. 1. Of the three systems, 5-OH-6-yl is the thermodynamically least stable one. The computed barriers and product stabilities again agree very well with B3LYP data obtained by using the same general methodology; the B3LYP activation free energies are in both OH addition cases 0.7 kcal/mol, and the products slightly less stable than the corresponding MPW1B95 data.

Also taking into account the complexation energies, as is shown in Figure 3, we note that the H-abstraction system is the least favorable initial complex to be formed, due to the stabilizing interaction between the hydroxyl oxygen and the π-cloud of the 5-MeCyt ring in the other two systems. This is also inferred from the relative similarity in the structures of the RC and TS for 5-OH-6-yl and for 6-OH-5-yl radicals, respectively, as well as from the very small changes in spin redistribution when reaching the TS (Figure 2c,d). Already in the RCs, 30−40% of the unpaired spin is transferred to the 5-MeCyt ring through this strong interaction. In the •OH radical addition products, the main component of the unpaired spin is found on the neighboring carbon atom, just as for the 5-H-6-yl and 6-H-5-yl radicals. The hydroxyl groups carry essentially no spin in the products. The 5-OH-6-yl addition process seems to be quite different from those observed for thymine.[19,20] We can attribute this difference to the NH_2 group, which has a completely different chemical behavior, due to a different electronic density, than the C═O group in thymine.

For the allylic system, there is throughout the reaction a strong hydrogen bonding interaction to one of the hydrogens of the N4 amino group, stabilizing the hydroxyl hydrogen to reside in the ring plane. In the RC, all unpaired spin is on the hydroxyl oxygen, whereas in the TS ca. 25% is transferred to 5-MeCyt (mainly the methyl group carbon). The TS is an early transition state, as manifested by the long O--H bond and short C−H bond. In the product, the resulting water molecule is still involved in a hydrogen bonding to the amine group, and the unpaired spin displays an alternant pattern between the methylene carbon, C6 and C5, with the main fraction on the methylene group.

The isotropic proton HFCCs of 5-OH-6-yl show a very similar pattern to the HC5 system, with a large negative α-coupling on H6. The small coupling on the hydroxyl proton is a result of the screening from the oxygen (i.e., that the proton is not attached directly to the ring). Also the 5-OH-6-yl radical is highly puckered, with the added OH group in the axial position. In the 6-OH-5-yl radical, the ring is less distorted than in the 5-OH-6-yl isomer, albeit still sufficiently nonplanar to render a relatively small β-coupling on the "more equatorial" H6. We emphasize, however, that we have not included vibrational averaging in the current study. The averaged methyl proton couplings are 56.8, in close agreement with the value for the H6-thymine adduct.

In the allylic 5-(cytosyl)methyl radical, finally, we have two large α-couplings on the methylene group, due to the larger spin component on this center, and a smaller negative α-coupling on H6. The agreement with available experimental data for various allylic 5-methylpyrimidine radicals, with different substituents at N1 and obtained at different temperatures or crystal compositions, is most satisfactory.

Again, the results obtained at the MPW1B95 level and the data from the corresponding solvent corrected B3LYP calculations are in close agreement — for the HFCCs the data agree to within a few percent.

Conclusions

In this work, we have studied the reaction mechanisms of the addition of •H and •OH radicals to the C5═C6 double bond of 5-methylcytosine. The calculations show that prereactive

species are formed with very low-energy barriers leading to the final products. Thermodynamically, the C6 addition is the most probable. As a secondary, but nonetheless very important reaction, the •OH induced proton abstraction from the methyl group was also studied. This reaction is the least favorable of the three reactions with •OH studied herein. The electron paramagnetic resonance hyperfine coupling constants (HFCC) have been determined in the DFT framework. The results are in very good agreement with the scarce experimental data available and also with those computed earlier for related compounds. To this end, we also evaluated reaction energetics and product radical HFCCs for the different cases, using the B3LYP functional (within the same methodological framework). The results are in close agreement, with B3LYP as expected giving slightly lower activation energies and exergonicity of the reactions. The HFCCs agree to within a few percent between the two methods.

This supports the validity of the method employed, and the reliability of the determination of the final adducts. The reaction pathways and the hyperfine coupling constants together give interesting insights to the formation processes and the conformational and spectroscopic properties of the •OH and •H mediated radical reaction products in pyrimidine nucleobases.

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References and Notes

(1) von Sonntag, C. *The Chemical Basis of Radiation Biology*; Taylor & Francis: London, UK, 1987.
(2) Steenken, S. *Chem. Rev.* **1989**, *89*, 503.
(3) Cadet, J.; Berger, M.; Douki, T.; Ravanat, J. L. *Rev. Physiol. Biochem. Pharmacol.* **1997**, *131*, 1.
(4) Breen, A. P.; Murphy, J. A. *Free Radical Biol. Med.* **1995**, *18*, 1033.
(5) Burrows, C. J.; Muller, J. G. *Chem. Rev.* **1998**, *98*, 1109.6.
(6) Cadet, J.; Sage, E.; Douki, T. *Mutat. Res.* **2005**, *571*, 3.
(7) Cadet, J.; Douki, T.; Gasparutto, D.; Ravanat, J.-L. *Radiat. Phys. Chem.* **2005**, *72*, 293.
(8) Castro, G. D.; Diaz Gomez, M. J.; Castro, J. A. *Chem. Biol. Interact.* **1996**, *99*, 289.
(9) Lion, Y.; van de Vorst, A. *Int. J. Radiat. Biol.* **1974**, *25*, 525.
(10) Pfeifer, G. P. *Mutat. Res.* **2000**, *450*, 155.
(11) Cier, A.; Lefier, A.; Ravier, M.-A.; Nofre, C. *C. R. Acad. Sci. Fr.* **1962**, *254*, 504.
(12) Kattak, M. N.; Green, J. H. *Int. J. Radiat. Biol.* **1966**, *11*, 137.
(13) Burdy, A.; Noyes, K. T.; Valinuck, V.; Sowers, L. C. *Nucleic Acids Res.* **2002**, *30*, 4068.
(14) Zhang, Q.; Wang, Y. *Nucleic Acids Res.* **2005**, *33*, 1593.
(15) Bienvenu, C.; Cadet, J. *J. Org. Chem.* **1996**, *61*, 2632.
(16) Bienvenu, C.; Wagner, J. R.; Cadet, J. *J. Am. Chem. Soc.* **1996**, *118*, 11406.
(17) Adamo, C.; Heitzmann, M.; Meilleur, F.; Grand, A.; Cadet, J.; Barone, V. *J. Am. Chem. Soc.* **2001**, *123*, 7113.
(18) Jolibois, F.; Grand, A.; Cadet, J.; Adamo, C.; Barone, V. *Chem. Phys. Lett.* **1999**, *301*, 255.
(19) Wetmore, S. D.; Boyd, R. J.; Llano, J.; Lundqvist, M. J.; Eriksson, L. A. In *Recent Advances in Density Functional Methods*; Barone, V., Bencina, A., Fantucci, P., Eds.; World Scientific: Singapore, 2002; Vol. 3.
(20) Jolibois, F. Thèse Doctorat es Sciences, Université Joseph Fourier, Grenoble, France, 1997.
(21) Morell, C.; Grand, A.; Barone, V.; Adamo, C. To be submitted for publication.
(22) Jolibois, F.; Cadet, J.; Grand, A.; Subra, R.; Rega, N.; Barone, V. *J. Am. Chem. Soc.* **1998**, *120*, 1864.
(23) Krivokapic, A.; Hole, E. I.; Sagstuen, E. *Radiat. Res.* **2003**, *160*, 340.
(24) Zhao, Y.; Truhlar, D. G. *J. Phys. Chem. A* **2004**, *108*, 6908.
(25) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskortz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chem, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, Revision A.1; Gaussian, Inc.: Pittsburgh, PA, 2003.
(26) Cancès, M. T.; Mennucci, B.; Tomasi, J. *J. Chem. Phys.* **1997**, *107*, 3032.
(27) Mennucci, B.; Tomasi, J. *J. Chem. Phys.* **1997**, *106*, 5151.
(28) Cossi, M.; Scalmani, G.; Rega, N.; Barone, V. *J. Chem. Phys.* **2002**, *117*, 43.
(29) Barone, V.; Adamo, C.; Grand, A.; Brunel, Y.; Fontecave, M.; Subra, R. *J. Am. Chem. Soc.* **1995**, *117*, 1083.
(30) Barone, V.; Adamo, C.; Grand, A.; Subra, R. *Chem. Phys. Lett.* **1995**, *242*, 351.
(31) Barone, V.; Adamo, C.; Grand, A.; Jolibois, F.; Brunel, Y.; Subra, R. *J. Am. Chem. Soc.* **1995**, *117*, 12618.
(32) Wetmore, S. D.; Eriksson, L. A.; Boyd, R. J. In *Theoretical Biochemistry−Processes and Properties of Biological Systems, Theoretical and Computational Chemistry*; Eriksson, L. A., Ed.; Elsevier: Amsterdam, The Netherlands, 2001; Vol. 9, pp 409−466 and referentes therein.
(33) Sagstuen, E.; Hole, E. O.; Nelson, W. H.; Close, D. M. *J. Phys. Chem.* **1992**, *96*, 1121.
(34) Sagstuen, E.; Hole, E. O.; Nelson, W. H.; Close, D. M. *J. Phys. Chem.* **1992**, *96*, 8269.
(35) Close, D. M.; Bernhard, W. A. *Bull. Am. Phys. Soc.* **1980**, *25*, 416.
(36) Cadet, J.; Douki, T.; Gasparutto, D.; Ravanat, J.-L. *Mutat. Res.* **2003**, *531*, 5.