J|A|C|S
COMMUNICATIONS

Electron Attachment-Induced DNA Single Strand Breaks: $C_{3'}-O_{3'}$ σ-Bond Breaking of Pyrimidine Nucleotides Predominates

Jiande Gu,*[†,‡] Jing Wang,[‡] and Jerzy Leszczynski*[,‡]

*Drug Design & Discovery Center, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica,
Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 201203,
People's Republic of China, and Computational Center for Molecular Structure and Interactions,
Department of Chemistry, Jackson State University, Jackson, Mississippi 39217*

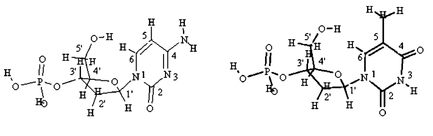
Received May 21, 2006; E-mail: jiandegush@go.com; jerzy@ccmsi.us

An understanding of the DNA strand breaks induced by low energy electrons (LEE) is of importance for the advancement of global models of cellular radiolysis and for the development of efficient methods of radiotherapy.[1] Both experimental investigations and theoretical studies of different DNA fragment models have demonstrated that, at near 0 eV energy, electron attachment may induce strand breaks in DNA.[2−14] Different DNA strand breaking mechanisms have been proposed in order to understand the nature of DNA strand breaks by LEEs.[7,9,11,14]

On the basis of the gas-phase model of the sugar−phosphate−sugar (S−P−S) moiety, Sevilla et al.[7] have proposed that LEE may be captured first by the phosphate, forming a phosphate-centered radical anion. Subsequent C−O σ-bond breaking was estimated to have an energy barrier of ∼10 kcal/mol. However, the studies of Simons et al. suggested a base-hosted anion mechanism and a much higher activation barrier (∼15 kcal/mol in the gas phase, ∼25 kcal/mol in an aqueous solution) for $C_{3'}-O_{3'}$ bond cleavage.[10−13,15] Moreover, recent experiments suggested that the DNA single strand breaks might be initiated by electron attachment to the bases in the gas phase.[16]

To elucidate the mechanism of DNA single strand breaks by LEEs, a reliable description of the properties of the radical anions of the nucleotides and the accurate determination of the activation energy barrier of the corresponding bond rupture is necessary. With the reliably calibrated DFT-based B3LYP/DZP++ approach, the electron affinity of 2′-deoxycytidine-3′-monophosphate and its phosphate deprotonated anion have been studied by Schaefer et al.[17] 3′-dCMPH is found to be able to capture near 0 eV electron to form a stable radical anion in both the gas phase and aqueous solution. With the same method, investigations of LEE attachment-induced $C_{5'}-O_{5'}$ σ-bond breaking of the pyrimidine nucleotides (5′-dCMPH and 5′-dTMPH) reveal that the activation barriers are about 14 kcal/mol in the gas phase and 18 kcal/mol in an aqueous solution.[18] In light of these findings, here we report theoretical investigations of LEE attachment-induced $C_{3'}-O_{3'}$ σ-bond breaking of pyrimidine nucleotides. 2′-Deoxycytidine-3′-monophosphate and 2′-deoxythymidine-3′-monophosphate in their protonated forms (3′-dCMPH and 3′-dTMPH, Scheme 1) have been selected as models. These models complement our previous studies for 5′-dCMPH and 5′-dTMPH.[18]

Scheme 1. Molecular Structures and Labels of the Models



3′-dCMPH **3′-dTMPH**

Scheme 2. The Proposed Mechanism of the LEE-Induced Single Strand Breaks

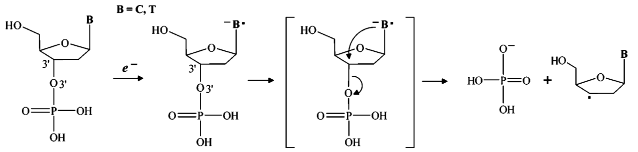


Table 1. Electron Affinities of 3′-dCMPH and 3′-dTMPH (in eV). The Values with Zero Point Correction Are Given in Parentheses

	EA_{ad}	VEA[a]	VDE[b]
3′-dCMPH → 3′-dCMPH⁻	0.33 (0.44)[c]	0.15[c]	1.28[c]
3′-dTMPH → 3′-dTMPH⁻	0.44 (0.56)	0.26	1.53
5′-dCMPH → 5′-dCMPH⁻	0.20 (0.34)[d]	−0.11[d]	0.85[d]
5′-dTMPH → 5′-dTMPH⁻	0.28 (0.44)[d]	0.01[d]	0.99[d]

[a] VEA = *E*(neutral) − *E*(anion); based on the optimized neutral structures. [b] VDE = *E*(neutral) − *E*(anion); based on the optimized anion structures. [c] From ref 17, and also this study. [d] From ref 18.

Following the proposed mechanism of the LEE-induced single strand bond breaking (SSB) in DNA (Scheme 2), the formation of the base-centered radical anions is the first step. Then, these anions undergo the C−O bond breaking and yield neutral ribose radical fragments and the phosphoric anions. The geometries of the neutral nucleotides, the corresponding radical anions, the transition states, and the $C_{3'}-O_{3'}$ σ-bond breaking products were fully optimized by the B3LYP/DZP++ approach.[19] Spin contamination has been found to be low (S^2 values are less than 0.757 before annihilation) for all the studied species. The GAUSSIAN 03 program[20] was used for all computations.

The adiabatic electron affinity (EA_{ad}) was evaluated to be 0.44 eV for 3′-dCMPH and 0.56 eV for 3′-dTMPH (Table 1). For comparison, the EA_{ad} values of 5′-dCMPH and 5′-dTMPH are 0.34 and 0.44 eV, respectively.[18] The vertical attachment energy (VEA) and the vertical detachment energy (VDE) amount to 0.26 and 1.53 eV for 3′-dTMPH, which are 0.11 and 0.25 eV larger than those of 3′-dCMPH.[17] These substantial positive EA values ensure that the corresponding radical anions are electronically stable even in the gas phase.

The transition states (located by using the "OPT = TS" option in the GAUSSIAN 03 program) for the $C_{3'}-O_{3'}$ σ-bond cleavage were characterized by the existence of the single imaginary vibrational frequency (598*i* cm^{-1} for 3′-dCMPH⁻ and 621*i* cm^{-1} for 3′-dTMPH⁻). The corresponding normal mode revealed by the analytical frequency calculation characterizes the $C_{3'}-O_{3'}$ σ-bond breaking (Figure 1). The $C_{3'}-O_{3'}$ atomic distance is 1.748 Å for 3′-dCMPH⁻ and 1.734 Å for 3′-dTMPH⁻, close to those predicted by Sevilla's group for the S−P−S model[7] and by the study for

---

[†] Chinese Academy of Sciences.
[‡] Jackson State University.

10.1021/ja063309c CCC: $33.50

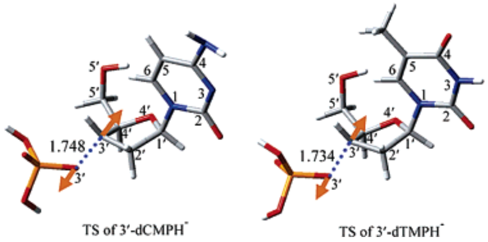


Figure 1. Optimized structures of the $C_{3'}-O_{3'}$ bond breaking transition states of 3′-dCMPH and 3′-dTMPH. Bond distances are in Å. Orange arrows represent the imaginary frequency-related vibration mode. Color representations are red for O, gray for C, blue for N, orange for P, and white for H.

Table 2. The Energy (in kcal/mol) of the Radical Anions, the Transition States, and the $C_{3'}-O_{3'}$ σ-Bond Broken Complexes of the Pyrimidine Nucleotides

	ΔE	$\Delta E^{0\,a}$	$\Delta G^{0\,a}$
3′-dCMPH$^-$	0.00	0.00	0.00
$TS_{3'\text{-dCMPH}^-}$	6.17 (12.82)[b]	4.68	4.54
cytidine-C3′(*HH*′)-yl	−20.81	−21.74	−24.43
+HOPO$_3$H$^-$	(−19.65)[b]		
3′-dTMPH$^-$	0.00	0.00	0.00
$TS_{3'\text{-dTMPH}^-}$	7.06 (13.73)[b]	5.29	4.42
thymidine-C3′(*HH*′)-yl	−20.22	−21.13	−23.70
+HOPO$_3$H$^-$	(−17.84)[b]		

[a] ΔE^0 = zero point energy corrected; ΔG^0 = free energy difference at 298 K. [b] Polarizable continuum model (PCM)[21] with $\epsilon = 78.39$.

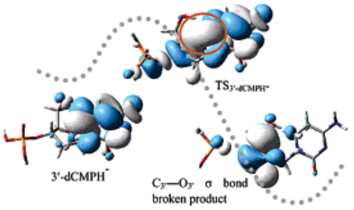


Figure 2. The SOMO of the electron attached state, the transition state, and the $C_{3'}-O_{3'}$ σ-bond broken product of 3′-dCMPH radical anion. The migration of the excessive negative charge through the atomic orbital overlap between the C_6 of pyrimidine and the $C_{3'}$ of ribose is shown in the orange circle.

5′-dCMPH and 5′-dTMPH.[18] Conversely, this distance is much longer (1.85−1.95 Å) at the Hartree−Fock level of theory.[12,13]

The most striking finding is the significantly low activation energy of the $C_{3'}-O_{3'}$ σ-bond cleavage (6.2 kcal/mol for 3′-dCMPH$^-$, 7.1 kcal/mol for 3′-dTMPH$^-$, Table 2). Such low activation energy consists with the experimental observation in electron induced DNA strand breaks.[2,16] For comparison, the energy barrier for the $C_{5'}-O_{5'}$ σ-bond rupture amounts to 14.3 and 13.8 kcal/mol for 5′-dCMPH and 5′-dTMPH, respectively.[18] Similar to the previous studies, the solvent effects increase the $C_{3'}-O_{3'}$ σ-bond cleavage barrier by up to 12.8 kcal/mol for 3′-dCMPH$^-$ and 13.7 kcal/mol for 3′-dTMPH$^-$, which are about 4−5 kcal/mol lower than the corresponding energy barrier for the $C_{5'}-O_{5'}$ σ-bond rupture.[18]

The examination of the singly occupied molecular orbital (SOMO) casts new light on the physical background of the low energy barrier of the $C_{3'}-O_{3'}$ cleavage. Figure 2 reveals that excessive charge on the base facilitates an attack on $C_{3'}$ from the back side of the leaving phosphate group. This resembles the nucleophilic S_N2 mechanism. The migration of the negative charge from the base to the $C_{3'}-O_{3'}$ bond proceeds directly through the atomic orbital overlap between the C_6 of pyrimidine and the $C_{3'}$ of ribose. Moreover, the characteristics of the antibonding orbital of the $C_{3'}-O_{3'}$ σ-bond shown in the SOMOs demonstrate the "charge-induced dissociation" feature.[14] The cooperative effects of the nucleophilic attack in the transition state of 3′-dCMPH and 3′-dTMPH further reduce the energy barrier of the $C_{3'}-O_{3'}$ σ-bond rupture.

Our results demonstrate that the transfer of the negative charge from the π^* orbital of the radical anion of pyrimidines to the DNA backbone does not pass through the N1-glycosidic bond. Instead, the migration of the excessive negative charge through atomic orbital overlap between the C_6 of pyrimidine and the $C_{3'}$ of ribose creates a highly promising pathway that subsequently leads to strand breaks. The proposed mechanism of the LEE-induced SSB assumes that the electronically stable radical anions may undergo either C−O bond breaking or N-glycosidic bond rupture. Previous research of pyrimidine nucleosides predicted an activation energy of 18.9−21.6 kcal/mol for the C−N bond break.[14] A recent study revealed that the energy barrier for the $C_{5'}-O_{5'}$ σ-bond rupture amounts to 13.8−14.3 kcal/mol.[18] The present investigation yields an energy barrier of 6.2−7.1 kcal/mol for $C_{3'}-O_{3'}$ σ-bond cleavage. Therefore, we conclude that the $C_{3'}-O_{3'}$ σ-bond rupture dominates the LEE-induced SSB of DNA.

Acknowledgment. Supported by Grants of ONR (N00034-03-1-0116), NSF CREST (HRD-0318519), and NIH SCORE (G1 2RR13459-21).

Supporting Information Available: Full citation for ref 20. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) LaVerne, J. A.; Pimblott, S. M. *Radiat. Res*. **1995**, *141*, 208.
(2) Boudaiffa, B.; Cloutier, P.; Hunting, D.; Huels, M. A.; Sanche, L. *Science* **2000**, *287*, 1658.
(3) Pan, X.; Cloutier, P.; Hunting, D.; Sanche, L. *Phys. Rev. Lett*. **2003**, *90*, 208102.
(4) Caron, L. G.; Sanche, L. *Phys. Rev. Lett*. **2003**, *91*, 113201.
(5) Hanel, G.; Gstir, B.; Denifl, S.; Scheier, P.; Probst, M.; Farizon, B.; Farizon, M.; Illenberger, E.; Mark, T. D. *Phys. Rev. Lett*. **2003**, *90*, 1881041.
(6) Zheng, Y.; Cloutier, P.; Hunting, D.; Wagner, J. R.; Sanche, L. *J. Am. Chem. Soc*. **2004**, *126*, 1002.
(7) Li, X.; Sevilla, M. D.; Sanche, L. *J. Am. Chem. Soc*. **2003**, *125*, 13668.
(8) Huels, M. A.; Boudaiffa, B.; Cloutier, P.; Hunting, D.; Sanche, L. *J. Am. Chem. Soc*. **2003**, *125*, 4467.
(9) Abdoul-Carime, H.; Gohlke, S.; Fischbach, E.; Scheike, J.; Illenberger, E. *Chem. Phys. Lett*. **2004**, *387*, 267.
(10) Barrios, R.; Skurski, P.; Simons, J. *J. Phys. Chem. B* **2002**, *106*, 7991.
(11) Berdys, J.; Anusiewicz, I.; Skurski, P.; Simons, J. *J. Am. Chem. Soc.* **2004**, *126*, 6441.
(12) Berdys, J.; Skurski, P.; Simons, J. *J. Phys. Chem. B* **2004**, *108*, 5800.
(13) Berdys, J.; Anusiewicz, I.; Skurski, P.; Simons, J. *J. Phys. Chem. A* **2004**, *108*, 2999.
(14) Gu, J.; Xie, Y.; Schaefer, H. F. *J. Am. Chem. Soc*. **2005**, *127*, 1053.
(15) Anusiewicz, I.; Berdys, J.; Sobczyk, M.; Skurski, P.; Simons, J. *J. Phys. Chem. A* **2004**, *108*, 11381.
(16) Martin, F.; Burrow, P. D.; Cai, A.; Cloutier, P.; Hunting, D.; Sanche, L. *Phys. Rev. Lett*. **2004**, *93*, 068101-1.
(17) Gu, J.; Xie, Y.; Schaefer, H. F. *J. Am. Chem. Soc*. **2006**, *128*, 1250.
(18) Bao, X.; Wang, J.; Gu, J.; Leszczynski, J. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 5658.
(19) Rienstra-Kiracofe, J. C.; Tschumper, G. S.; Schaefer, H. F.; Nandi, S.; Ellison, G. B. *Chem. Rev*. **2002**, *102*, 231.
(20) Frisch, M. J.; et al. *Gaussian 03*, version c.02; Gaussian, Inc.: Pittsburgh, PA, 2004.
(21) Cossi, M.; Barone, V.; Cammi, R.; Tomasi, J. *Chem. Phys. Lett*. **1996**, *255*, 327.

JA063309C