DNA Abasic Sites

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Findings on the Electron-Attachment-Induced Abasic Site in a DNA Double Helix**

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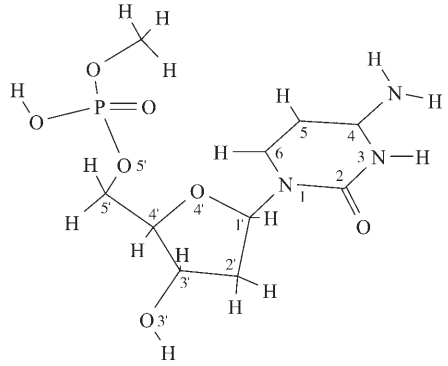
A detailed knowledge of DNA damage induced by low-energy electrons (LEE) are of crucial importance both for the advancement of theoretical models of cellular radiolysis and for the development of new methods of radiotherapy.[1] Studies on various DNA fragments have demonstrated that near 0 eV energy, electron attachment may induce strand breaks in DNA.[2–15] Strand-breaking mechanisms have been proposed to elucidate the nature of DNA damage by LEE.[7,9–15] Both experimental and theoretical studies suggest that the base-hosted radical anions might be responsible for the LEE-induced DNA single-strand breaks.[3,9,11,13–15] These electronically stable radical anions (covalent-bonded anions)[13–15] are capable of undergoing either C–O rupture or glycosidic bond breaking (forming an abasic site). However, as far as cytosine is concerned, this pathway might be retarded in double-stranded DNA. Although the pairing between cytosine (C) and guanine (G) dramatically increases the electron affinity of C (from 0.1 eV for C to 0.4 eV for a GC pair),[16,17] a minor activation barrier (≈ 4 kcal mol^{-1}, which is lower than the activation energy (≈ 6 kcal mol^{-1}) for the LEE-induced C3′-O3′ bond breaking in DNA single strands[15]) for proton transfer (PT) from the N1 of G to the N3 of C in the GC pair might neutralize the negative charge of the cytosine (forming a C(N3H)$^{\bullet}$ neural radical) before possible cleavage reactions.[17,18]

Notably, the nucleotide of the C(N3H)$^{\bullet}$ neural radical might accommodate an extra electron, forming a closed-shell anionic nucleotide of C(N3H)$^{-}$ ion. A recent study demonstrated that intramolecular proton transfer from the C2′ of ribose to the C6 of the C(N3H)$^{-}$ ion might trigger the $C_{3'}$–$O_{3'}$ σ bond cleavage.[19] In light of these findings, we report theoretical investigations of the LEE-induced abasic site at the 3′ end of double-stranded DNA. Neutral 2′-deoxycytidine-5′-monophosphate with the N3 protonated cytosine radical was adopted as a starting model in our study (denoted as [5′-dC(N3H)MPH]$^{\bullet}$) because C(N3H)$^{\bullet}$ is a main LEE-induced product involved in the GC pair.[17,18] For a better description of the influence of the 3′–5′ phosphodiester linkage in DNA, the -OPO_3H moiety was terminated with a methyl group (see Scheme 1). This model represents the N3 protonated cytidine at the 3′ end of a DNA strand.



Scheme 1. The molecular model showing the N3-protonated cytidine at the 3′ end of a DNA strand ([5′-dC(N3H)MPH]$^{\bullet}$).

The geometries of local minima and corresponding transition states were fully optimized at the B3LYP/DZP++ level,[20] which has been proven to be a reliable approach for describing the structures and energetics of radicals and anions related to the DNA components.[12–16,21,22] The GAUSSIAN 03 program[23] was used for all computations.

Based on optimized structures (Figure 1), the adiabatic electron affinity (EA_{ad}) is evaluated to be 0.40 eV for [5′-dC(N3H)MPH]$^{\bullet}$ (Table 1), which is 0.06 eV larger than that of 5′-dCMPH (0.34 eV).[14] The vertical attachment energy (VEA) amounts to −0.21 eV and the vertical detachment energy (VDE) amounts to 5.56 eV for [5′-dC(N3H)MPH]$^{\bullet}$. Moreover, the VDE of the corresponding anion [5′-dC(N3H)MPH]$^{-}$ is predicted to be 1.02 eV. For comparison, the VEA of 5′-dCMPH is −0.11 eV and the VDE of the corresponding radical anion [5′-dCMPH]$^{-}$ is 0.85 eV.[14] The large VDE and the small negative VEA of [5′-dC(N3H)MPH]$^{\bullet}$ ensure the high probability of the LEE attachment. Meanwhile, the substantial positive EA_{ad} and VDE values indicate that the [5′-dC(N3H)MPH]$^{-}$ anion is electronically stable. It should be noted that owing to the electrostatic repulsion, the EA_{ad} of [5′-dC(N3H)MPH]$^{\bullet}$ is expected to be greatly reduced when it closely interacts with

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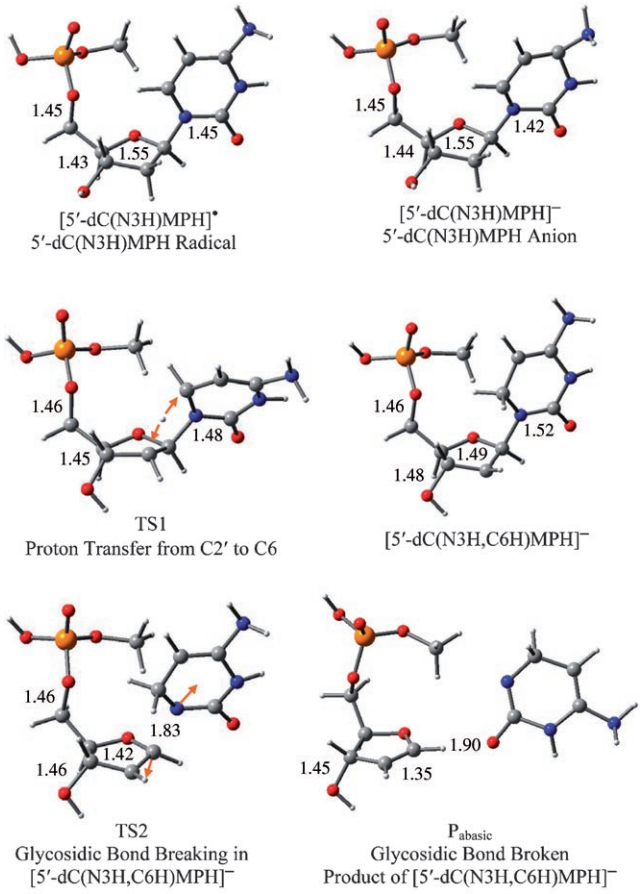


Figure 1. Optimized structures and the corresponding transition states. Bond distances are in Å. Orange arrows in the transition states represent the single imaginary-frequency-related vibration mode. O red, C gray, N blue, P orange, and H white.

the deprotonated G anion in the gas phase. However, recent studies have shown that the EA_{ad} of nucleotides are nearly independent of the existence of counterions in aqueous solution.[13,24] Therefore, the electrostatic repulsion from the deprotonated G anion in aqueous solution should not lower the EA_{ad} of [5′-dC(N3H)MPH]•. Our additional calculations demonstrate that the EA_{ad} of [5′-dC(N3H)MPH]• in aqueous solution amounts to 1.46 eV when it is bounded to the deprotonated G anion.

Table 1: Electron attachment and detachment energies for [5′-dC(N3H)MPH]•.[a]

	EA_{ad} [eV]	VEA[b] [eV]	VDE[c] [eV]
[5′-dC(N3H)MPH]•→ [5′-dC(N3H)MPH]+			5.56
[5′-dC(N3H)MPH]•→ [5′-dC(N3H)MPH]−	0.32 (0.40)	−0.21	1.02
5′-dCMPH→5′-dCMPH−	0.20 (0.34)[d]	−0.11[d]	0.85[d]

[a] The values corrected for zero point energy are given in parentheses. [b] VEA = $E_{anion} - E_{neutral}$, the energy is evaluated based on the optimized neutral structure. [c] VDE = $E_{neutral} - E_{anion}$ for the [5′-dC(N3H)MPH]− ion, the energy is evaluated based on the optimized anion structure; and VDE = $E_{cation} - E_{neutral}$ for [5′-dC(N3H)MPH]•, the energy is evaluated based on the optimized neutral structure. [d] Reference [20].

The intramolecular proton transfer from the C2′ of ribose to the C6 of cytosine within the [5′-dC(N3H)MPH]− ion results in the local minimal structure, [5′-dC(N3H, C6H)MPH]−, which is more stable than the original anion by 3.3 kcal mol^{-1}. The inspection of the corresponding transition state reveals that the activation energy barrier for the PT (from C2′ to C6) is 13.2 kcal mol^{-1}. The activation energy of 5 kcal mol^{-1}, which was predicted for the similar process in the [3′-dC(N3H)MPH]− anion,[19] indicates that PT is sensitive to the presence of the phosphate group at the neighboring O3′ position. In fact, PT induces the C3′–O3′ bond rupture[19] in the [3′-dC(N3H)MPH]− ion, whereas it leads to a local minimum [5′-dC(N3H, C6H)MPH]− ion for the [5′-dC(N3H)MPH]− ion (Table 2).

Notably, the glycosidic bond (N1–C1′) elongates remarkably in the [5′-dC(N3H, C6H)MPH]− ion as compared with that of the [5′-dC(N3H)MPH]− ion (1.58 versus 1.46 Å). Alternatively, the elongation of the C3′–O3′ and C5′–O5′ bonds is insignificant (0.02 and 0.01 Å, respectively). Accordingly, the N1–C1′ bond rupture is expect to dominate the transformation of the [5′-dC(N3H, C6H)MPH]− ion.

The transition state for the glycosidic bond breaking is characterized by the significantly increased N1–C1′ distance (1.83 Å) and the considerably reduced C1′–C2′ bond length (1.42 Å). The examination of the vibrational mode corresponding to the single imaginary frequency confirms the N1–C1′ bond breaking. The activation energy for the N1–C1′ bond rupture is evaluated to be 2.7 kcal mol^{-1}. Previous research on the LEE-induced glycosidic bond cleavage in pyrimidine nucleosides predicted the energy barrier to be as high as 21.6 kcal mol^{-1} for cytidine.[12] The negative charge transfer from the base to the ribose (accompanied by the PT) greatly weakens the glycosidic bond.

The product of the glycosidic bond cleavage in the [5′-dC(N3H, C6H)MPH]− ion, a complex with the abasic nucleotide and the released cytosine (P_{abasic} in Figure 1), is more stable than the original anion by 17.8 kcal mol^{-1}. The C1′–C2′ bond distance at the abasic site exhibits double bond character (1.35 Å) and the natural population analysis shows that the negative charge resides on the base moiety.

Thus, electron attachment to the N3-protonated cytidine at the 3′ end of a DNA strand might lead to the formation of an abasic site according to the mechanism presented in Scheme 2.

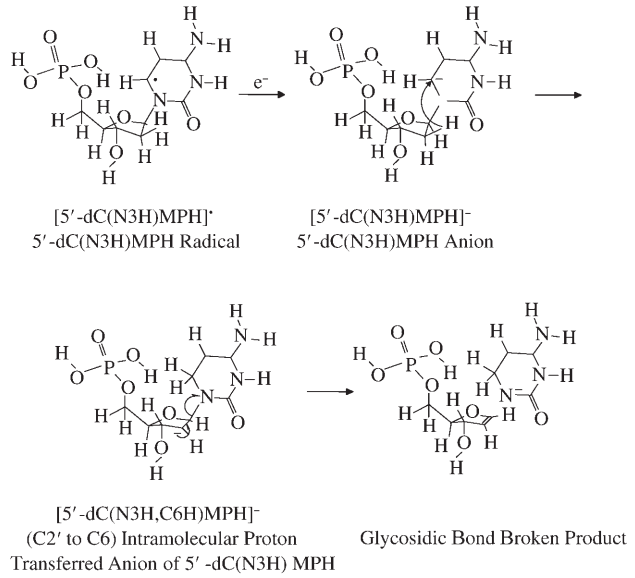
The potential energy surface along the pathway of the formation of the abasic site depicted in Figure 2 demonstrates

Table 2: The relative energy (ΔE), ΔE corrected for zero-point energy (ΔE^0), and free energy at 298 K (ΔG^0) for stationary points on the reaction pathway.[a]

	ΔE [kcal mol^{-1}]	ΔE^0 [kcal mol^{-1}]	ΔG^0 [kcal mol^{-1}]
[5′-dC(N3H)MPH]−	0.00	0.00	0.00
TS1	13.16	9.87	12.18
[5′-dC(N3H, C6H)MPH]−	−3.26	−2.67	−1.68
TS2	−0.49	−0.90	0.22
P_{abasic}	−17.77	−18.95	−21.67

[a] The energies are calculated at the B3LYP/DZP++ level.

that the rate-controlling step of the depyrimidine (cytidine) at the 3′ end of DNA strands is the intramolecular PT from the C2′ of ribose to the C6 of cytosine. The activation energy of this rate-controlling step is 13.2 kcal mol^{-1}, about 14 kcal mol^{-1} lower than the VDE of the [5′-dC(N3H)MPH]$^-$ ion (≈1 eV or 23 kcal mol^{-1}). Therefore, electron detachment is not expected to reduce the possibility of the formation of the abasic site.



Scheme 2. The proposed mechanism for the formation of an abasic site caused by LEE attachment to the N3-protonated cytidine at the 3′ end of a DNA strand.

In summary, the present study along with previous investigations[16–18] suggest that LEE might induce the formation of an abasic site at the 3′ end of a DNA double helix with a strand ended with a cytidine residue. Strong thermodynamic stimulus for the overall process and a low kinetic barrier of the rate-controlling step indicate that the LEE attachment to the DNA helix might significantly contribute to the radiation-induced DNA damage.

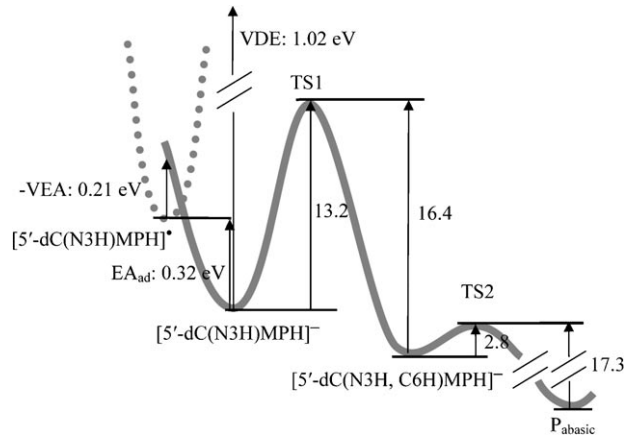


Figure 2. The potential-energy surface along the pathway leading to the formation of the abasic site (P_{abasic}). The energy is in kcal mol^{-1}, except when otherwise indicated.

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