# Hydrogen Atom Loss in Pyrimidine DNA Bases Induced by Low-Energy Electrons: Energetics Predicted by Theory

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In addition to inducing DNA strand breaks, low-energy electrons (LEEs) also have been shown to induce fragmentation of pyrimidine bases (uracil, thymine, and cytosine) in the gas and condensed phases. Loss of a hydrogen atom from a DNA base−electron adduct initiates chemical modification of the base, which can cause permanent damage to the base as well as to DNA. Thus, the energetics of hydrogen atom loss reactions from anionic bases is crucial to understanding the mechanism of LEE-induced damage to DNA and its component bases. Following our previous report on LEE interactions with uracil [*J. Phys. Chem. B* **2004**, *108*, 5472−5476], in this work we investigate LEE interactions with thymine and cytosine. The adiabatic potential energy surface along each N−H or C−H bond is explored up to 3 Å at the DFT level. The changes in energy, enthalpy, and free energy ($\Delta E$, $\Delta H$, and $\Delta G$) for a complete separation of an H atom or a methyl (amino) group from the anionic base as well as bond dissociation energies of neutral bases are calculated at the CBS-Q level. The electron affinities of the DNA base thymine and cytosine and their H-deleted neutral fragments are also calculated. All N−H bonds are more susceptible to LEE-induced fragmentation than C−H bonds, with $N_1$−H as the most vulnerable site. Since $N_1$ is the site of the glycosidic bond between the deoxyribose and the base in DNA, the vulnerable nature of this site toward bond rupture suggests that LEEs are likely to induce base release in DNA. Investigations along these lines are under way.

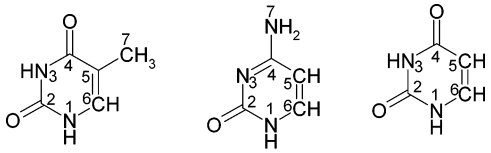
## Introduction

Recent experiments[1−8] have uncovered the fact that relatively low energy electrons (LEEs) can effectively induce fragmentation of DNA/RNA's *pyrimidine* bases, such as thymine, cytosine, and uracil, by dissociative electron attachment (DEA).[9] These experiments clearly show that LEEs are capable of initiating chemical modifications of DNA/RNA bases; i.e., LEEs likely cause specific DNA damage and enhance radiation-induced mutations. Although most experiments were performed in the gas phase, there is good evidence that such fragmentations also occur in the condensed phase, and are likely important in DNA damage in a living cell in which it may lead to point mutations. For example, recent results in condensed-phase DNA indicate that 3−20 eV LEEs result in electron-stimulated ejection of fragment anions such as $H^-$, $O^-$, and $OH^-$.[10] And it has been suggested that the $H^-$ originated mainly from the bases, rather than the deoxyribose rings.[10] Electron-stimulated formation of $H^-$ from gaseous deoxyribose[11] and from its condensed-phase analogues[12] has also been reported. It was confirmed that hydrogen loss is not the predominant reaction channel for LEE-induced fragmentation of deoxyribose.[11]

Ionizing radiation leaves along its track a large number of free electrons with kinetic energies below ∼20 eV. Since LEEs carry a large portion of radiation energy deposited in the medium, and they are the most abundant secondary species in radiolysis, the contribution of LEEs to DNA damage, including strand breaks and fragmentation of DNA components, may have been underestimated. For example, it has been shown that LEEs can induce the most lethal form of radiation-induced DNA damage, i.e., strand breaks,[13−19] and modifications to the bases which may result in transmission of the altered genetic code via replication.

We have recently reported[20] a theoretical investigation of the energetics of LEE interaction with uracil. It was found that[20] dissociation of H from any of the N−H and C−H bonds of uracil anion is endothermic; the calculated adiabatic potential energy surfaces suggest an energy threshold for formation of hydrogen from N−H and C−H bonds in the order 0.78 ($N_1$) < 1.3 ($N_3$) < 2.2 ($C_6$) < 2.7 eV ($C_5$). The H-deleted uracil radicals have exceptionally high adiabatic electron affinities, i.e., 3.46 ($N_1$), 3.8 ($N_3$), 2.35 ($C_5$), and 2.67 eV ($C_6$). These high electron affinities reduce the energy needed to break the N−H or C−H bonds and provide an explanation for the large hydrogen yield found experimentally from uracil upon attachment of LEEs.[3,5]

The present study is an extension of our efforts to employ theoretical calculations to understand the fragmentations of the pyrimidine bases (thymine, cytosine) induced by LEEs. The potential energy surfaces along the N−H or C−H bonds of the pyrimidine base anions, as well as the energetics for the fragmentation process, are presented. The structures and numbering schemes of these three bases are shown below:

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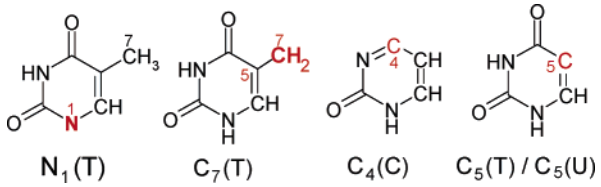
Fragment species considered include all one hydrogen atom loss species from the above structures. The nomenclature employed in this work for these structures is the site of the hydrogen atom loss with the base in parentheses; for example, see $N_1$(T) and $C_7$(T) below for hydrogen atom loss from $N_1$ and $C_7$ on thymine. Similarly, for group loss the site of the loss is given with the base in parentheses; for example, see $C_4$(C) below for the loss of an amine group from $C_4$ of cytosine and $C_5$(T) for loss of the methyl group from thymine [same as $C_5$-(U)].




## Methods

Details of the methods have been described in previous papers.[20] Briefly, most calculations were performed for isolated structures in the gas phase with the Gaussian 98 program package.[21] The B3LYP functionals with the 6-31+G(d) basis set are chosen as a minimal DFT standard method for geometry optimizations, frequency analysis, and adiabatic potential energy surface (PES) searches. More detailed discussions about the reliability of this level of the theory are available in refs 22−24 including references therein. It must be pointed out that, at this level of theory, there is no diffuse bound "*dipole bound*" state contribution to the equilibrium anionic states of thymine, cytosine, or uracil.[23]

The equilibrium anion state of cytosine is an interesting case. Optimization starting from a planar input geometry at the B3LYP/6-31+G(d) level usually will lead to a structure at an energy of −394.9374378 au, with three negative imaginary frequencies. The optimization was repeated using Gaussian 03 B05[25] and Spartan '04,[26] besides Gaussian 98 A7, and all gave the same result. However, starting from a nonplanar geometry similar to that in an optimized anionic guanine−cytosine base pair (see ref 27) gives an energy of −394.9438307 au, without an imaginary frequency. This geometry is "puckered" and is used as a starting point for subsequent calculations. For more details about the anionic states of cytosine, see ref 28.

Adiabatic PESs along the C−H or N−H bond stretch were calculated using the optimization keyword opt=ModRedundant, with the S action code in the additional input, which performs geometry optimization for each point along the specified range of C−H/N−H distances, from ∼1 up to 3.0 Å with a 0.05 Å step size.

More accurate energy calculations were performed at the CBS-Q[29] level for various structures obtained by the DFT method. CBS-Q calculations give excellent results for bond dissociations and electron affinities with average errors reported for bond dissociations as less than 1 kcal/mol (maximum ca. 2.3 kcal/mol) for a large range of systems.[29] Detailed discussion on the accuracy of this method can be found in ref 30.

## Results and Discussion

**Potential Energy Surfaces.** The energetic profile of a bond-breaking process can be best described by the PES along the bond. For the bond breaking of pyrimidine bases induced by low-energy electron attachment, a full investigation of the PESs along the bonds of the resulting anions renders a clear picture of the energetics of bond fragmentation.

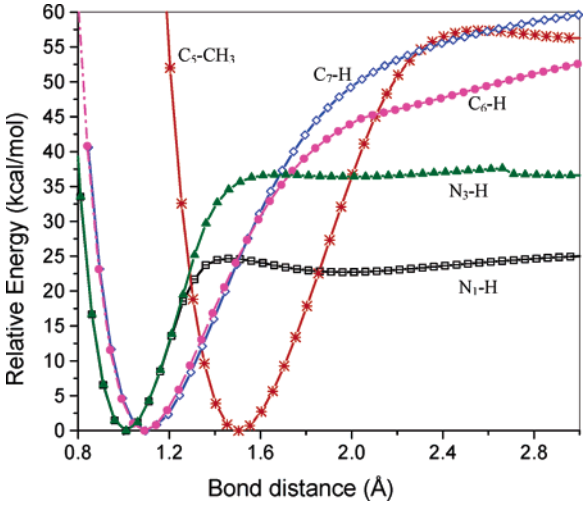



**Figure 1.** Adiabatic potential energy surfaces of the thymine anion radical along each N−H or C−H coordinate, calculated at the B3LYP/6-31+G(d) level. Energy relative to that of the optimized anion in the equilibrium state. The zero-point energy is not included.

In the case of thymine, the adiabatic PES of its anion (Figure 1) along each of the N−H or C−H bonds suggests that cleavage of the $N_1$−H bond requires the least energy, followed by the $N_3$−H, the $C_6$−H, and the methyl C−H bonds. Most of the energy required to break the N−H bonds is spent in the initial 1.1−1.4 Å. Beyond 1.4 Å, the N−H PES is relatively flat. This indicates that, once the N−H bond is stretched to over 1.4 Å, the bond is essentially ruptured. This is not the case for C−H bonds, and this difference reflects differences in the electronic states in the base fragment which accept the electron.

Thus, the electronic states of the anionic radicals are critical to understanding the N−H and C−H bond scission process. The PES begins with the molecule in the $\pi^*$ state and ends in the antibonding $\sigma^*$ state as described in our previous work on uracil.[20] For the N−H bond at 1.4 Å, the system crosses from the $\pi^*$ state to the $\sigma^*$ state. The stability of the final $\sigma^*$ state depends on the electron affinities of the site of attachment. Since the carbon sites have lower electron affinities than the nitrogen sites, fragmentation of a C−H bond needs substantially more energy than that of an N−H bond. The difference in energy in the PESs between the minimum and large internuclear distances represents the bond dissociation energy (BDE) of the molecular anion radical, BDE(molecular anion). This value can be found from the bond energy of the neutral parent molecule, BDE-(parent), and the electron affinities of the neutral parent molecule, EA(parent), and the base radical fragment, EA-(radical), as indicated by the equations below for thymine (T):

$$T \rightarrow T(-H)^{\bullet} + H^{\bullet} \qquad \text{BDE(parent)}$$

$$T(-H)^{\bullet} + e^{-} \rightarrow T(-H)^{-} \qquad \text{EA(radical)}$$

$$T^{-} \rightarrow T + e^{-} \qquad \text{EA(parent)}$$

$$T^{-} \rightarrow T(-H)^{-} + H^{\bullet} \qquad \text{BDE(molecular anion)}$$

Thus

$$\text{BDE(anion)} = \text{BDE(parent)} + \text{EA(parent)} - \text{EA(radical)} \quad (1)$$

Note that according to the EA sign convention used is this work, a positive EA represents a negative potential (i.e., a bound electron). Since the electron affinities of the neutral "parent" molecules are near zero, a good estimate of the bond energy of the anion radical is the difference in the bond energy of the parent and the electron affinity of the fragment species, BDE-(molecular anion) = BDE(parent) − EA(radical) (see sections below for the values of the bond dissociation energy and electron affinities).

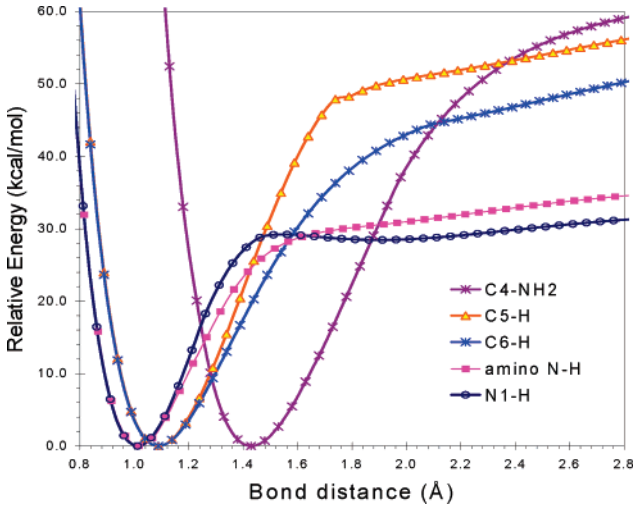



**Figure 2.** Adiabatic potential energy surfaces of the cytosine anion radical along each N−H or C−H coordinate, calculated at the B3LYP/6-31+G(d) level. Energy relative to that of the optimized anion in the equilibrium state. The zero-point energy is not included.

The shapes of the PESs suggest that the nitrogen−hydrogen $\sigma^*$ state is considerably antibonding, whereas some bonding or at least substantially less antibonding character is suggested for the C−H bond in its $\sigma^*$ state. This may be because the electron affinities of the carbon site radicals are more comparable to that of the hydrogen atom, resulting in more three-electron bond contribution than that for the NH bond. In agreement with this contribution, spin distributions show that spin sharing between the atoms in the bond is maintained to longer distances for C−H bonds than for N−H bonds (see ref 20).

It is also clear that hydrogen atom loss from the pyrimidine ring is easier than from the methyl group. Indeed, on the basis of the energies involved, hydrogen atom loss from the methyl group is less likely than cleavage of the whole methyl group. The energy needed for hydrogen atom loss is in the order $N_1 < N_3 < C_6 < C_7$ (methyl group). The PES along the $C_5-CH_3$ bond (Figure 1) appears to experience a transition maximum of ∼57 kcal/mol, and then goes slightly downward. Further separation of thymine anion into uracil-5-yl anion plus a $\bullet CH_3$ neutral fragment leads to an identical $\Delta E$ of +57 kcal/mol, calculated at the CBS-Q level, showing an unfavorable energetics for the thymine anion to fragment at the $C_5-CH_3$ position.

The PESs of anionic cytosine are shown in Figure 2. On the basis of these PESs, it can be seen that hydrogen atom loss requires about 20 kcal/mol less energy from the N centers than from the C centers, with the $N_1$ site requiring the least energy and the $C_5$ site the most, and the energy needed for hydrogen atom loss is in the order $N_1 < N_7$ (amino) $< C_6 < C_5$. This order of the PESs is similar to that of thymine (Figure 1) and uracil.[20] The $C_4-NH_2$ PES in Figure 2 shows that removing the amino group ($-NH_2$) from the cytosine anion requires at least 60 kcal/mol of energy, which is even larger than that required for removing the methyl group from the thymine anion.

Interestingly, the patterns of anionic PESs of thymine are quite similar to those of uracil[20] previously reported, at the three $N_1-H$, $N_3-H$, and $C_6-H$ bonds. Figure 3 directly compares the anionic PESs of thymine, cytosine, and uracil at their $N_1-H$ or $C_6-H$ bonds. Thymine and uracil have very similar PESs along the $N_1-H$ bond, which are about 5 kcal/mol lower in relative energy than that of cytosine at longer distance of the $\sigma^*$-type portion. The PESs along the $C_6-H$ bonds of thymine and uracil almost overlap each other, and lie slightly higher, above that of cytosine at longer bond distance position. Since the $N_1-H$ bond PES is the lowest, $N_1$ is the most probable site for LEE-induced hydrogen atom loss to occur in each base; thus, the relative energy cost of hydrogen atom loss reaction at the $N_1$ site may be correlated with the absolute cross section of the dehydrogenated fragment. The comparison of $N_1-H$ PESs in Figure 3 suggests that the $N_1-H$ bond of thymine or uracil is more susceptible to fragmentation induced by LEEs, than that of cytosine. This may explain the experimental observation[4] of a lower cross section of the hydrogen-loss negative fragment of cytosine $(C - H)^-$, $2.3 \times 10^{-16}$ cm$^2$,[4] than thymine $(T - H)^-$, $1.2 \times 10^{-15}$ cm$^2$,[3] and uracil $(U - H)^-$, $3 \times 10^{-16}$cm$^2$.[3]

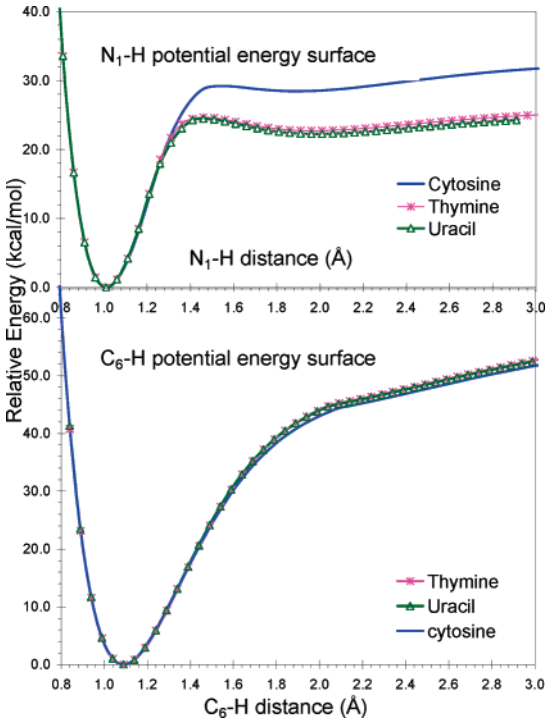



**Figure 3.** Comparison of the PESs for the three pyrimidine-base anion radicals, calculated at the B3LYP/6-31+G(d) level. The upper figure compares the $N_1-H$ PESs and the lower figure shows $C_6-H$ PES similarity of thymine, cytosine, and uracil.

**Energetics for Bond Breaking.** The potential energy surfaces calculated at the DFT level have qualitatively shown the energetics order for hydrogen atom loss from a pyrimidine base after attachment of a low-energy electron (see Figures 1−3). Quantitatively, the DFT method also predicts energy changes for such a loss with good quality, high efficiency, and relatively low computational cost. More accurate theoretical values of the energetics, however, need deployment of a higher level of theory, which requires more extensive computational resources to improve the accuracy from ±3 kcal/mol with the DFT method to ca. ±1 kcal/mol with, for example, the CBS-Q method.[29−30]

Table 1 lists the energy changes, $\Delta H$ and $\Delta E$, for infinite separation of a hydrogen atom or a methyl group from the thymine anion at 298 K. In addition, the BDEs of neutral thymine at each site are also listed. Table 2 lists these values for the cytosine anion. Two sets of data are provided for each process, one calculated at the B3LYP/6-31+G(d) level and the other calculated with the CBS-Q method. As can be seen from Table 1, removing a hydrogen atom from the N positions of the thymine anion costs only about half the energy needed for the C positions, with the $N_1$ position needing the least energy (20 kcal/mol). Surprisingly, removing the whole methyl group requires less energy (CBS-Q value 57 kcal/mol) than removing a hydrogen on the methyl group (63 kcal/mol, position $C_7$). On

**TABLE 1: Infinite Separation: $T^- \rightarrow (T - H)^- + H$[a]**

| | $N_1$ | $N_3$ | $C_6$ | $C_7$ | $C_5-CH_3$[b] |
|---|---|---|---|---|---|
| | | | B3LYP/6-31+G(d) | | |
| $\Delta E$, 0 K | 20.32 | 32.67 | 52.55 | 63.81 | 52.90 |
| $\Delta E$, 298 K | 20.54 | 33.05 | 52.82 | 64.42 | 53.39 |
| $\Delta H$ | 21.13 | 33.64 | 53.41 | 65.01 | 53.98 |
| $\Delta G$ | 15.00 | 27.12 | 47.27 | 58.03 | 42.62 |
| BDE(T),[c] 0 K | 91.24 | 114.86 | 109.30 | 85.25 | 103.53 |
| BDE(T),[c] 298 K | 92.15 | 115.92 | 110.32 | 85.90 | 104.46 |
| | | | CBS-Q | | |
| $\Delta E$ | 20.45 | 30.73 | 50.35 | 63.37 | 57.03 |
| $\Delta H$ | 21.05 | 31.32 | 50.94 | 63.97 | 57.62 |
| $\Delta G$ | 14.59 | 24.51 | 44.42 | 57.48 | 46.78 |
| BDE(T)[c] | 97.25 | 124.4[d] | 113.47 | 87.65 | 113.35 |

[a] All calculations were done for the gas phase and $T = 298$ K except as indicated. All values are given in kilocalories per mole. [b] $T^- \rightarrow (T - CH_3)^- + \bullet CH_3$. [c] Directly calculated bond dissociation energies for neutral thymine. [d] CBS-Q calculation failed; therefore, this value was calculated from eq 1.

**TABLE 2: Infinite Separation: $C^- \rightarrow (C - H)^- + H$[a]**

| | $N_1$ | $C_5$ | $C_6$ | $N_7$ (amino N−H) | $C_4-NH_2$[b] |
|---|---|---|---|---|---|
| | | | B3LYP/6-31+G(d) | | |
| $\Delta E$, 0 K | 25.98 | 57.44 | 49.88 | 27.95 | 71.32 |
| $\Delta E$, 298 K | 27.26 | 60.13 | 51.71 | 30.54 | 72.09 |
| $\Delta H$ | 27.36 | 58.85 | 51.24 | 29.54 | 72.68 |
| $\Delta G$ | 19.69 | 51.15 | 43.69 | 21.22 | 60.74 |
| BDE,[c] 0 K | 96.50 | 111.71 | 107.15 | 99.56 | 100.69 |
| BDE,[c] 298 K | 97.35 | 112.83 | 108.21 | 100.16 | 101.50 |
| | | | CBS-Q | | |
| $\Delta E$, 298 K | 27.48 | 60.11 | 51.76 | 30.66 | 75.61 |
| $\Delta H$ | 28.08 | 60.70 | 52.36 | 31.26 | 76.20 |
| $\Delta G$ | 21.18 | 53.62 | 45.53 | 23.90 | 64.97 |
| BDE,[c] 298 K | N/A | 116.81 | 111.68 | 108.18 | 104.69 |

[a] All calculations were done for the gas phase and $T = 298$ K except as indicated. All values are given in kilocalories per mole. [b] $C^- \rightarrow (C - NH_2)^- + \bullet NH_2$. [c] Bond dissociation energies for neutral cytosine.

the basis of the data listed in Table 1, it can be concluded that hydrogen atom loss from thymine induced by LEEs should mainly occur on the $N_1$ and $N_3$ positions. The possibility for hydrogen atom loss is in the order $N_1 > N_3 \gg C_6 > C_7$ (methyl). Since transition maxima are not obvious in the PESs (Figure 1), the $\Delta E$ listed in Table 1 is the upper limit of the energy threshold for each reaction. Obviously the $N_1$ position is the weakest point for LEE-induced fragmentation. It is interesting to note that the $\Delta E$ values in Table 1 are generally in good agreement with those reported by Denifl et al.,[4] except that for the $N_1$ position they found a value of 1.7 eV, or 39 kcal/mol, using the B3LYP method, which is obviously different from our value of 20.3 kcal/mol in Table 1, and from their own values of 0.8−0.9 eV obtained by the G2MP2 method.

In thymidine, $N_1$ is the site of the glycosidic bond to the deoxyribose; since N−C bonds are weaker than N−H bonds, it is clear that the $N_1$ bond would remain a weak link. To test this, we employed the $N_1-CH_3$ bond in $N_1$-methylthymine anion radical as a simple model for glycosidic bond fragmentation. We find that $\Delta E = 2.28$ kcal/mol, $\Delta H = 2.87$ kcal/mol, and $\Delta G = -7.88$ kcal/mol (298 K) for $N_1-CH_3$ bond scission at the B3LYP/6-31+G(d) level. This is substantially lower than the energetics of $N_1-H$ scission in Table 1 and confirms the susceptibility of this bond to fragmentation. Recent experimental reports have confirmed that LEEs can induce glycosidic bond cleavage in thymidine.[31,32] Within DNA, the 3′-C−O and 5′-C−O bonds that couple the phosphate to the DNA backbone have also been shown in theoretical work to be quite vulnerable to LEE-induced fragmentation.[17,18] A theoretical study of LEE-induced glycosidic bond fragmentation is now under way.

**TABLE 3: Comparison of $\Delta E$ for Bond Cleavage in Pyrimidine Anion Radicals Calculated at the CBS-Q Level (kcal/mol)**

| position | uracil | thymine | cytosine |
|---|---|---|---|
| $N_1$ | 19.47 | 20.45 | 27.48 |
| $C_6$ | 51.36 | 50.3 | 51.76 |

For the cytosine anion, hydrogen atom loss from the $N_1$ and even the amino group positions also needs much less energy than from the $C_5$ and $C_6$ positions (Table 2), and $N_1$ remains the most vulnerable site to hydrogen loss. The possibility for hydrogen atom loss from the cytosine anion will be in the order $N_1 >$ amino $\gg C_6 > C_5$. Major hydrogen atom loss fragmentation from cytosine induced by LEEs is favored on the N positions. Unlike thymine, where demethylation is more favorable than hydrogen atom loss from the methyl group, deamination is most unlikely to occur for the gas-phase cytosine anion.

The two sets of data listed in Tables 1 and 2 for each reaction are intended for a comparison of the accuracy between the DFT and the CBS-Q methods. In general, both data sets are in reasonably good agreement. For hydrogen atom loss, the predictions made by the DFT B3LYP method are very close to those obtained by CBS-Q, with the largest difference being within 3 kcal/mol. For demethylation in thymine (Table 1) and deamination processes, the differences between the two methods are slightly larger, but still not exceeding 4.5 kcal/mol. Note that there is no systematic bias in all three terms ($\Delta E$, $\Delta H$, and $\Delta G$), i.e., all values (such as all $\Delta E$ values) by the DFT method are not always higher (or always lower) than those calculated by CBS-Q. The CBS-Q method usually predicts energetics of a reaction to within 1 kcal/mol of the experimental values. For bond dissociation energies of the neutral parent molecules, the CBS-Q results are systematically higher than the DFT results by 6 kcal/mol on average. Overall, the comparison suggests that the DFT method is ideal for estimation of the energetics of heterolytic bond dissociations in the pyrimidine anion radicals, but gives somewhat low values for homolytic bond cleavages in the neutral molecules.

With the data in Tables 1 and 2, and those previously reported for uracil,[20] it is also possible to compare the three pyrimidine bases for hydrogen loss induced by LEEs at the $N_1$ or $C_6$ positions. For convenience, these $\Delta E$ values are collected in Table 3. It can be seen that, for hydrogen loss at the $N_1$ position induced by LEE attachment, uracil needs the least amount of energy (19.47 kcal/mol), thymine needs slightly more (20.45 kcal/mol), and cytosine requires the largest amount of energy (27.48 kcal/mol). For hydrogen loss to occur at the $C_6$ position, the three pyrimidine bases need similar large amounts of energy (∼50 kcal/mol). These values are calculated at the CBS-Q level and are considered among the best theoretical predictions. They confirm the tendency shown on the PESs calculated at the DFT level (Figures 1−3). Since $N_1$ is the most vulnerable site in each base, the $\Delta E$ values for $N_1$ listed in Table 3 confirm the conclusion from comparison of $N_1-H$ PESs in Figure 3; i.e., the $N_1-H$ bond of thymine or uracil is more susceptible to fragmentation induced by LEEs than that of cytosine. This agrees with the experimental observation[4] that the cross section of the cytosine dehydrogenated anionic fragment $(C - H)^-$ is much lower than those of thymine $(T - H)^-$ and uracil $(U - H)^-$.[3]

**Electron Affinities of the Radicals Formed by Stripping One H Atom from Thymine or Cytosine.** The high electron affinities of H-deleted radicals of the pyrimidine bases are

**TABLE 4: Electron Affinities of the Bases and Their H-Deleted Radicals (eV)[a]**

| | B3LYP/6-31+G(d) | | CBS-Q | |
|---|---|---|---|---|
| | no ZPE | +ZPE | 0 K | 298 K |
| thymine | 0.0164 | 0.145 | −0.0417 | −0.0593 |
| $N_1$(T)[b] | 3.23 | 3.22 | 3.26 | 3.27 |
| $N_3$(T) | 3.74 | 3.71 | 4.00[c] | 4.00[c] |
| $C_6$(T) | 2.60 | 2.61 | 2.67 | 2.68 |
| $C_7$(T)[c] | 1.00 | 1.08 | 0.99 | 0.99 |
| cytosine | −0.16 | −0.055 | −0.127 | −0.131 |
| $N_1$(C) | 2.91 | 2.90 | 3.58 | 3.58 |
| $C_5$(C) | 2.18 | 2.21 | 2.32 | 2.34 |
| $C_6$(C) | 2.34 | 2.33 | 2.41 | 2.44 |
| $N_7$(C)[d] | 2.96 | 2.96 | 3.23 | 3.23 |
| $C_4$(C)[e] | 1.15 | 1.13 | 1.28 | 1.27 |
| uracil[20] | | | | 0.002 |
| $N_1$(U) | 3.48 | 3.46 | | 3.49 |
| $N_3$(U) | 3.82 | 3.78 | | 4.16 |
| $C_5$(U) | 2.30 | 2.34 | | 2.38 |
| $C_6$(U) | 2.68 | 2.67 | | 2.68 |

[a] All the anion radicals treated are valence anions. The column heads indicate the thermal correction (i.e., no ZPE, +ZPE, 0 K, and 298 K). [b] Parent base indicated in parentheses. [c] CBS-lq is used instead of CBS-Q since the latter failed. [d] Fragment resulting from H atom loss from the methyl group for thymine and amino group for cytosine. [e] Fragment from loss of the complete $C_4$ amino group.

believed to be the major factor that enables LEE-induced hydrogen atom loss to occur.[3−5,20] These high electron affinities reduce the energy needed to break the N−H or C−H bonds and provide an explanation for the large hydrogen yield found experimentally from attachment of LEEs to uracil,[3] thymine, and cytosine.[2,4−6]

Table 4 lists the adiabatic electron affinities (AEA) of various fragmental radicals from one-H-deleted thymine or cytosine. Note that these electron affinities refer to the valence anions. The DFT B3LYP calculated EAs are listed together with those of the CBS-Q method for comparison, but the discussion below refers to the CBS-Q results at 298 K, unless specified otherwise. The CBS-Q calculated values for uracil fragments previously reported were also included in the last column to facilitate comparison. The electron affinity of $C_4$(C), which is the fragment of cytosine with the amino group at $C_4$ removed, is also included in Table 4. Except for the $C_4$(C) fragment, all the radicals result from deleting one H from different positions on thymine or cytosine, and the side group (H or amino) deleted site is indicated in parentheses. For example, $N_1$(T) means the fragment of thymine with H on $N_1$ removed, and $N_7$(C) means the cytosine fragment radical with an amino H removed.

As can be seen in Table 4, for the thymine H-deleted radicals, the EAs are in the order $N_3 > N_1 > C_6 \gg$ methyl (CBS-Q results), and for those of cytosine, the order is $N_1 >$ amino $> C_6 > C_5$. The N-centered radicals have a substantially higher EA than the C-centered radicals, even if the N-center is the amino group in the case of cytosine. In contrast, the methyl(T) radical, which is a carbon-centered radical on the $CH_2$• group, has an EA as low as 1 eV. As a result, the loss of hydrogen from the methyl group of anionic thymine is greatly endothermic (63.3 kcal/mol, Table 1). Interestingly, the $C_4$(C) radical, which results from loss of the amino group in cytosine, has a low EA of 1.27 eV. The process of separating the amino group from anionic cytosine needs an energy of 75.6 kcal/mol (Table 2), which is the largest among all the reactions listed in Tables 1 and 2.

Among the three bases, for the $N_1$-centered radical, $N_1$(C) has the highest EA, followed by $N_1$(U) and then $N_1$(T). And for the $C_6$-centered radical, the T's and U's have the same EA of 2.68 eV, but that of the C's is slightly lower at 2.44 eV. The overall order of EAs calculated with the CBS-Q method for all the radicals is $N_3$(U) > $N_3$(T) > $N_1$(C) > $N_1$(U) > $N_1$(T) > $N_7$(C) > $C_6$(U) = $C_6$(T) > $C_6$(C) > $C_5$(U) > $C_5$(C) ≫ $C_4$(C) > $C_7$(T).

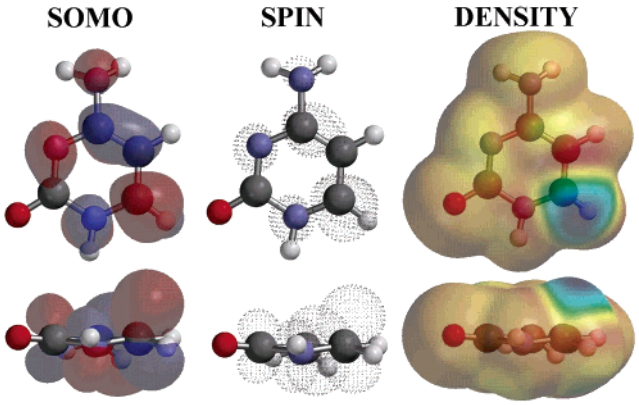



**Figure 4.** SOMO, electron spin density distribution, and total electron density of the equilibrium cytosine anion employing the B3YP/6-31+G-(d) method. Contour levels employed are SOMO at 0.032, spin at 0.002, and total electron density at 0.002 with spin density mapped onto the surface. All surfaces were visualized using Spartan '04 (Wavefunction, Inc.).[26]

We note that the radicals formed by hydrogen atom or group loss from each of the positions on the bases are all localized $\sigma$ radicals with the exception of the radical produced by loss of a hydrogen from the methyl group of thymine ($C_7$(T)), which is an allylic $\pi$ radical. While this may explain the latter's unusually small EA, we note that $C_4$(C) also has an unusually low EA, but is a $\sigma$ radical.

The DFT B3LYP results are in fairly good agreement with those of CBS-Q for radicals of thymine, except that, for thymine itself, the DFT method predicts a positive EA of 0.14 eV, but the CBS-Q method gives a small negative EA of −0.059 eV. The best estimates from experiment suggest values of EA closer to the CBS-Q calculation.[33] In the case of cytosine, the two methods are in acceptable agreement for the C-centered radicals, but the DFT method predictions provide an EA 0.3−0.6 eV lower than that from the CBS-Q method for the N-centered radicals. These discrepancies may arise from geometry optimization, since the B3LYP geometry for DFT and MP2 geometry in CBS-Q calculations differ. It is not possible to say which calculations are superior where large differences occur. Although experimental measurements could give the answer, higher level calculations would be helpful.

One concern with cytosine is that it has a significant negative electron affinity (see Table 4), which suggests a possible mixing of diffuse states with valence states in its equilibrium anion. Visualization of cytosine anion's singly occupied molecular orbital (SOMO), spin, and electron density shows no indication of diffuse states (see Figure 4). This ensures that the starting cytosine anion radical is in a valence state in the case of the B3LYP calculations. Note however, that an extensive discussion of the valence and diffuse anion radical states of cytosine can be found in ref 28.

There is a remaining question about the fragmentation products, i.e., whether an H atom or an anionic $H^-$ results. In the previous report on uracil,[20] it has been shown by following the spin density and charge variations with bond distance that such fragmentation leads to an H atom plus the remaining anionic fragment. The intrinsic preference for an H atom over $H^-$ is due to the exceptionally higher electron affinity of each

of the neutral uracil fragments than the H atom. The high electron affinities shown in Table 4 for the fragments of thymine and cytosine clearly indicate the energetic preference for H atom loss during fragmentation of thymine or cytosine anion radicals. As expected the calculations of spin density performed in this work confirm that the H atom is lost in each case. On the other hand, $C_4$(C) has an unusually low EA of 1.27 eV (CBS-Q result), but this is still higher than the EA of the amino group radical ($\bullet NH_2$), which is 0.73 eV. Thus, for the lowest energy path the $C_4$(C) radical acquires the electron during the fragmentation. Clearly in an energetic cleavage both $NH_2^-$ and $\bullet NH_2$ would be produced. For thymine the methyl radical ($\bullet CH_3$) is found to have a small but negative EA (−0.084 eV, CBS-Q result), so in the cleavage of the methyl group from the thymine anion, the methyl group leaves as a neutral radical while the electron is acquired by the $C_5$(T) radical.

## Summary

The interactions of LEEs with DNA's pyrimidine bases, thymine and cytosine, have been experimentally shown to induce hydrogen atom loss via a "dissociative electron attachment" mechanism. Current theoretical efforts to characterize the PESs and energetics of these hydrogen atom loss processes yield the following conclusions.

(1) N−H bonds are more vulnerable than C−H bonds toward LEE-induced fragmentation in all pyrimidine bases, with the $N_1$−H bond most vulnerable.

(2) Anions of thymine and uracil have very similar PESs, along both $N_1$−H and $C_6$−H, and their $N_1$−H PESs lie below that of cytosine's anion at extended bond distances. This may be the major reason the cross section of the cytosine dehydrogenated anionic fragment $(C - H)^-$ is much lower than those of thymine $(T - H)^-$ and uracil $(U - H)^-$.[3]

(3) Thymine, cytosine, and uracil have near-zero electron affinity. CBS-Q calculations predict the valence $\pi^*$ states are slightly unstable (negative EAs) and have a nonplanar geometry (see the Supporting Information for the dihedrals of this geometry). However, DFT theory suggests that thymine and uracil have a slightly positive EA. Experiments at this point favor the CBS-Q values and suggest that the DFT results are slightly too high.[33] As the N−H or C−H bond in the valence $\pi^*$ states is stretched to longer distances, the anion shifts to the planar $\sigma^*$ state. For the N−H bond PES, such a shift occurs at around 1.4 Å, and most of the spin is transferred to the H atom beyond this distance; while for the C−H bond PES, such a shift starts at around 1.6 Å and the spin is gradually transferred to the departing hydrogen atom at long distances.

(4) The H-deleted fragments of thymine, cytosine, and uracil have high electron affinities which effectively reduce the energy cost of N−H or C−H bond breaking from the anions. Specifically, electron affinities of the nitrogen-centered fragments are substantially higher than those of the carbon-centered fragments. For this reason, it is expected that LEE-induced hydrogen atom loss from these pyrimidine bases will be mostly from N−H bonds, and this is found experimentally.

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**Supporting Information Available:** Optimized geometry of the fragment neutral radicals and their anions (TXT). This material is available free of charge via the Internet at http://pubs.acs.org.

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