Electron attachment-induced DNA single-strand breaks at the pyrimidine sites

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ABSTRACT

To elucidate the contribution of pyrimidine in DNA strand breaks caused by low-energy electrons (LEEs), theoretical investigations of the LEE attachment-induced C3–O3 σ bond as well as N-glycosidic bond breaking of 2'-deoxyctydine-3',5'-diphosphate and 2'-deoxythymidine-3',5'-diphosphate were performed using the B3LYP/DZP++ approach. The base-centered radical anions are electronically stable enough to assure that either the C–O or glycosidic bond breaking processes might compete with the electron detachment and yield corresponding radical fragments and anions. In the gas phase, the computed glycosidic bond breaking activation energy (24.1 kcal/mol) excludes the base release pathway. The low-energy barrier for the C3–O3σ bond cleavage process (∼6.0 kcal/mol for both cytidine and thymidine) suggests that this reaction pathway is the most favorable one as compared to other possible pathways. On the other hand, the relatively low activation energy barrier (∼14 kcal/mol) for the C5–O5σ bond cleavage process indicates that this bond breaking pathway could be possible, especially when the incident electrons have relatively high energy (a few electronvolts). The presence of the polarizable medium greatly increases the activation energies of either C–O σ bond cleavage processes or the N-glycosidic bond breaking process. The only possible pathway that dominates the LEE-induced DNA single strands in the presence of the polarizable surroundings (such as in an aqueous solution) is the C3–O3σ bond cleavage (the relatively low activation energy barrier, ∼13.4 kcal/mol, has been predicted through a polarizable continuum model investigation). The qualitative agreement between the ratio for the bond breaks of C5–O5, C3–O3 and N-glycosidic bonds observed in the experiment of oligonucleotide tetramer CGAT and the theoretical sequence of the bond breaking reaction pathways have been found. This consistency between the theoretical predictions and the experimental observations provides strong supportive evidences for the base-centered radical anion mechanism of the LEE-induced single-strand bond breaking around the pyrimidine sites of the DNA single strands.

INTRODUCTION

Both recent experimental and theoretical investigations of different DNA models have illustrated that low-energy electrons (LEE) play a vital role in the nascent stage of DNA radiolysis and may induce strand breaks in DNA via dissociative electron attachment (1–23). A comprehensive understanding of such LEE-induced DNA damages is one of the key steps towards governing the effects of ionizing radiation at a molecular level. Theoretical investigations on the mechanism of the LEE-induced DNA have been mainly focused on the pyrimidine families of DNA fragments (6,13,16,18,21,23). Based on the density functional theory (DFT) studies of the sugar–phosphate–sugar model, Li, Sevilla and Sanche (6) proposed that the near 0 eV electron may be captured first by the phosphate group, forming a phosphate-centered radical anion. More detailed study suggested that the excess electron is trapped in the dipolar field of two OH groups in the sugar–phosphate backbone (24). The subsequent C3–O3 or C5–O5σ

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bond breaking was estimated to have an energy barrier of \(\sim 10\) kcal/mol. Other theoretical studies (10) suggested that electrons with kinetic energies near 0 eV cannot directly attach to the phosphate units at a significant rate. The small values of electron affinity \([-0.003 \text{ and } 0.033 \text{ eV (6)}]\) of the evaluated sugar–phosphate–sugar model seem to suggest that, instead of the phosphate group in DNA species, LEEs might be trapped in the pyrimidine bases [with electron affinities of near 0 eV in experiments (25), and 0.03 eV (cytosine) and \(\sim 0.2\) eV (thymidine) at the B3LYP/DZP++ level of theory (26)]. Recent experimental and theoretical investigations of the base-releasing process of pyrimidine nucleosides (8,13,16,18,21) have suggested that at the nascent stage, the excess electron resides on the \(\pi^*\) orbital of pyrimidine in the radical anion, forming an electronically stable radical anion. The subsequent bond breaking might happen at either the C–O \(\sigma\) bond or N-glycosidic bond.

Based on the studies of different models \(2'\text{-deoxyctydine-3'-monophosphate and } 2'\text{-deoxy-thymidine3'-monophosphate molecule; (9–12,19)}\), Simons suggested that only in an aqueous solution, the very LEEs can attach to the \(\pi^*\) orbitals of the DNA bases and then undergo C\(_3\)-O\(_3\) bond cleavage (9,11,12,19). Negative electron affinities of the pyrimidine nucleotides in the gas phase, predicted in their studies, prevent the electron attachment to the bases. However, this conclusion does not agree with the experimental gas-phase investigations. The negative values for electron affinity of the pyrimidine nucleotides in the gas phase (9–11) are contrary to the experimental results on DNA (24) and RNA (4) fragments. Both experiments and other higher level theoretical investigations provide definitely the positive electron affinities for the pyrimidine bases, the nucleosides, and the nucleotides in the gas phase (13,26–28). Moreover, experiments on DNA strand breaks induced by 0–4 eV electrons suggest that the strand breaks are initiated by electron attachment to the bases in the condensed phase (29). Other studies suggested that the excited states might play an important role in LEE-induced strand breaks in DNA in aqueous solutions (30,31).

The density functional theory investigations of LEE-induced C–O \(\sigma\) bond breaking of pyrimidine nucleotides based on various pyrimidine-monophosphate models (16,18,23) concluded that the mechanism of the LEE-induced single-strand bond breaking in DNA involves the attachment of an electron to the pyrimidine bases of DNA and the formation of base-centered radical anions even in the gas phase. These radical anions might subsequently undergo either C–O or glycosidic bond breaking, yielding neutral ribose radical fragments and the corresponding phosphoric anions or base anions. The C\(_3\)-O\(_3\) bond cleavage is expected to dominate because of its low activation energy.

The elegantly selected models in the previous reaction pathway studies of LEE-induced DNA strand breaking have covered the main components of the DNA strands. It should be noted that in these studies three different bond ruptures have been investigated separately based on different models. The influences of the neighboring fragments on the bond breaking process have been neglected. For instance, phosphate group at 5’-position has not been taken into consideration in the models for the studies of the C\(_3\)-O\(_3\) or N-glycosidic bond breaking. However, recent experiment demonstrates that the terminal phosphates affect significantly the LEE-induced strand breakages of DNA oligomers (32). Moreover, theoretical investigation on the electron attachment to the nucleoside-3',5'-diphosphate suggests considerable influences of the phosphate group on the electron affinities (33). Therefore, a more complex model containing phosphate groups at both 3'- and 5'-positions of nucleoside is necessary for a more realistic elucidation of the mechanism of the damage at the pyrimidine sites in the DNA single strand by LEEs.

We report the first study of the reaction pathways of the LEE-induced pyrimidine-related DNA bond breakings of 2'-deoxyctydine-3',5'-diphosphate (3',5'-dCDP) and 2'-deoxythymidine-3',5'-diphosphate (3',5'-dTDP). Such a model allows simultaneous examination of both C\(_3\)-O\(_3\) and C\(_5\)-O\(_5\) bond cleavages and N-glycosidic bond rupture processes. (For a better description of the 3'-5' phosphodiester linkage in DNA, and to avoid the unrealistic intramolecular proton transfer from the phosphate group at the 5'-position to the base, the \(\text{-PO}_3\text{H}\) moiety was terminated with \(\text{CH}_3\) group; Figure 1). This conformation complements and enhances the previous studies of the monophosphate ester of the 2'-deoxyribonucleosides of pyrimidines, and provides information directly related to the important building blocks of DNA. In living systems, the phosphates of the nucleotides could be either negatively charged or neutralized by counterions. The neutral phosphate models used in this study represent situation in which counterions are closely bound to the phosphate group of DNA. However, the finding in the previous study (34) that the electron affinities of the nucleotides are independent of the counterions in aqueous solutions ensures the existence of electronically stable base-centered radical anions of nucleotides and provides support for the currently considered models.

**METHOD OF CALCULATION**

The DFT method employing B3LYP functional (35,36) with basis sets of double-\(\zeta\) quality augmented by polarization and diffuse functions (denoted DZP++) was used to obtain optimized geometries, energetics and natural charges for the DNA subunits in both neutral and anionic forms. The DZP++ basis sets were constructed by augmenting the Huzinaga–Dunning (37–39) set of contracted double-\(\zeta\) Gaussian functions. To complete the DZP++ basis, one even-tempered diffuse \(s\) function was added to each \(H\) atom, while sets of even-tempered diffuse \(s\) and \(p\) functions were centered on each heavy atom. The even-tempered orbital exponents were determined according to the recommendation of Lee and Schaefer (40).

The B3LYP functional has successfully reproduced the experimentally derived electron affinities of nucleobases.
(26,41) and also had accurately predicted the electron affinities of other DNA subunits such as nucleosides (27), which have been confirmed later by experiments (42). In addition, this functional provides a reliable description of the properties of the radical anions of the nucleotides and the reasonable determination of the activation energy barrier of the corresponding bond rupture (16,18,21,23). In accord with these previous successful applications, the B3LYP/DZP++ level of theory was also used in the present study.

To evaluate the potential energy surfaces of bond ruptures of DNA single strands in aqueous solution, a polarizable continuum model [PCM; (43)] with dielectric constant of water (ε = 78.39) was used to simulate the solvated environment of an aqueous solution. It should be noted that this PCM model approximate the real situation of aqueous solvation only to some extent, because the important effects of the microsolvation could not be included in this approach. Rather, the PCM model used in the present study accounts for the existence of the polarizable surroundings, which resembles situations in the experiment of LEE-induced bond breaks in the thin solid films. Natural Population Analysis was carried out using the mentioned functional and the DZP++ basis set with the Natural Bond Orbital analysis of Reed and Weinhold (44,45). The Gaussian 03 (46) system of DFT programs (Revision E. 01, 2004; Gaussian, Wallingford, CT, USA) was used for all computations.

Table 1. Electron attachment and detachment energies (in eV)

<table>
<thead>
<tr>
<th>Process</th>
<th>EA_{ad}</th>
<th>VEA^a</th>
<th>VDE^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>3',5'-dCDP → 3',5'-dCDP^−</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gas phase</td>
<td>0.27 (0.44)c</td>
<td>0.03c</td>
<td>0.71c</td>
</tr>
<tr>
<td>3',5'-dTDP → 3',5'-dTDP^−</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gas phase</td>
<td>0.35 (0.52)c</td>
<td>0.17c</td>
<td>0.67c</td>
</tr>
<tr>
<td>3',5'-dCDP → 3',5'-dCDP^−</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCM model</td>
<td>1.99c</td>
<td>1.45c</td>
<td>2.22c</td>
</tr>
<tr>
<td>3',5'-dTDP → 3',5'-dTDP^−</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCM model</td>
<td>1.98c</td>
<td>1.57c</td>
<td>2.17c</td>
</tr>
</tbody>
</table>

Numbers within the parentheses are the zero-point vibrational energy corrected.

^aVEA = E(neutral) – E(anion); the energies are evaluated using the optimized neutral structures.

^bVDE = E(neutral) – E(anion); the energies are evaluated using the optimized anion structures.

Gu et al. (33).

RESULTS AND DISCUSSION

Electron affinities of the nucleotides

The electron attachment and detachment energies of 3',5'-dTDP and 3',5'-dCDP are listed in the Table 1. These values are the same as those reported in the previous work (30). The adiabatic electron affinity (EA_{ad}) of 0.27 eV for 3',5'-dCDP and 0.35 eV for 3',5'-dTDP favor the formation of the corresponding radical anions. Meanwhile, the large values of the vertical detachment energy (VDE) for these two radical anions (0.71 eV for 3',5'-dCDP^− and 0.67 eV for 3',5'-dTDP^−) ensure that, in the gas phase, electron detachment will not compete with the subsequent reactions.
with the activation energy barrier $<16.37 \text{kcal/mol}$ (0.71 eV) for $3',5'$-dCDP$^{--}$ and $<15.45 \text{kcal/mol}$ (0.67 eV) for $3',5'$-dTDP$^{--}$.

Solvent effects remarkably increase the electron capturing ability of the nucleoside diphosphates. The $E_{\text{ads}}$s are 1.99 eV and 1.98 eV for $3',5'$-dCDP$^{--}$ and $3',5'$-dTDP$^{--}$ respectively, in the PCM calculation. Moreover, the increased VDE of $3',5'$-dCDP$^{--}$ (2.22 eV) and $3',5'$-dCTP$^{--}$ (2.17 eV) suggests that in aqueous solution the reactions with energy barrier less than 50 kcal/mol might undergo without electron detachment from these radical anion.

**Activation energies of the C$_5$–O$_5$ σ bond breaking**

The transition state structures for the C$_5$–O$_5$ σ bond cleavage process of the $3',5'$-dCDP$^{--}$ and $3',5'$-dTDP$^{--}$ have been located on the potential energy surface. These transition states are characterized by the existence of a single imaginary vibrational frequency (934/cm for $3',5'$-dCDP$^{--}$ and 956/cm for $3',5'$-dTDP$^{--}$). The C$_5$–O$_5$ σ bond breaking can be documented by the elongated C$_5$–O$_5$ atomic distance of 1.777 Å for cytidine (1.769 Å for thymidine) and by the analysis of normal mode corresponding to the imaginary vibrational frequency (Figures 1 and 2). The activation energy of the C$_5$–O$_5$ σ bond cleavage process has been predicted to be 14.17 kcal/mol for $3',5'$-dCDP$^{--}$ and 13.37 kcal/mol for $3',5'$-dTDP$^{--}$ [Table 2; without the zero point energy (ZPE) correction]. These values are very close to the activation energy needed for the C$_5$–O$_5$ σ bond breaking in 5′-dCMPH$^{--}$ (14.27 kcal/mol) and in 5′-dTMPH$^{--}$ (13.84 kcal/mol) (16). Table 2 also lists the ZPE-corrected activation energy barriers and the corresponding free-energy differences at 298 K. Since these values are close to the activation energy barriers without the ZPE correction (within 2 kcal/mol), the following discussions will mainly be based on the results without the ZPE correction.

The solvent effects increase the C$_5$–O$_5$ σ bond breaking energy barrier dramatically. The energy barriers predicted using the PCM model are 18.73 kcal/mol for $3',5'$-dCDP$^{--}$ and 18.76 kcal/mol for $3',5'$-dTDP$^{--}$ (Table 3). This noticeable increase of the energy barrier is close to that found for the pyrimidine monophosphate models (17.97 kcal/mol for 5′-dCMPH$^{--}$ and 17.86 kcal/mol for 5′-dTMPH$^{--}$) in the presence of polarizable medium (16).

**Activation energies of the C$_3$–O$_3$ σ bond breaking**

The transition states for C$_3$–O$_3$ σ bond cleavage process in the radical anion of $3',5'$-dCDP and $3',5'$-dTDP are characterized by the elongated C$_3$–O$_3$ atomic distance (1.738 Å) and the normal mode (with the C$_3$–O$_3$ σ bond breaking pattern; Figures 1 and 2) corresponding to the imaginary vibrational frequency. The activation energy of the C$_3$–O$_3$ σ bond breaking has been predicted to be 6.02 and 6.37 kcal/mol for the radical anions (Table 2; without ZPE). This energy barrier is similar to that reported based on the $3'$-dCMP and $3'$-dTMP models (6.17 kcal/mol for the former and 7.06 kcal/mol for the latter) at the same level of theory (18). The presence of the phosphate group

![Figure 2.](https://example.com/figure2.png)
The transition state for N-radical anion has been located and characterized by the activation energies of the solvent–solute interactions. C3(SN2)-like mechanism observed in the gas phase for the phase. Therefore, bimolecular nucleophilic substitution a and the corresponding normal mode representing the relative energies of transition states of bond break pathways in gas phase (kcal/mol)

<table>
<thead>
<tr>
<th>Bond breaking</th>
<th>ΔE_{TS}^a</th>
<th>ΔE_{TS}^{0}</th>
<th>ΔG_{TS}^{0}</th>
</tr>
</thead>
<tbody>
<tr>
<td>3′,5′-dCDP−</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C5–O2 bond</td>
<td>14.17 (14.27)</td>
<td>12.31 (12.52)</td>
<td>13.53 (12.75)</td>
</tr>
<tr>
<td>C3–O2 bond</td>
<td>6.03 (6.17)</td>
<td>5.23 (4.68)</td>
<td>7.60 (4.54)</td>
</tr>
<tr>
<td>N-glycosidic bond</td>
<td>26.21 (21.6)</td>
<td>24.95 (20.4)</td>
<td>26.57 (21.2)</td>
</tr>
<tr>
<td>3′,5′-dTDP−</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C5–O2 bond</td>
<td>13.39 (13.84)</td>
<td>11.59 (11.91)</td>
<td>11.49 (11.82)</td>
</tr>
<tr>
<td>C3–O2 bond</td>
<td>6.04 (7.06)</td>
<td>5.66 (5.29)</td>
<td>6.92 (4.42)</td>
</tr>
<tr>
<td>N-glycosidic bond</td>
<td>19.19 (18.9)</td>
<td>18.79 (17.6)</td>
<td>21.10 (18.0)</td>
</tr>
</tbody>
</table>

ΔE_{TS} = E(Transition state) – E(Radical anion).
ΔE_{TS}^{0} = ΔE_{TS} + ZPE correction.
ΔG_{TS}^{0} = ΔE_{TS} + ZPE correction.

Table 3. The relative energies of transition states of bond break pathways in aqueous solutions (kcal/mol)

<table>
<thead>
<tr>
<th>Bond breaking process</th>
<th>ΔE_{TS}^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>3′,5′-dCDP−</td>
<td></td>
</tr>
<tr>
<td>C5–O2 bond</td>
<td>18.73 (17.97)</td>
</tr>
<tr>
<td>C3–O2 bond</td>
<td>13.36 (12.82)</td>
</tr>
<tr>
<td>N-glycosidic bond</td>
<td>26.34</td>
</tr>
<tr>
<td>3′,5′-dTDP−</td>
<td></td>
</tr>
<tr>
<td>C5–O2 bond</td>
<td>18.76 (17.86)</td>
</tr>
<tr>
<td>C3–O2 bond</td>
<td>14.18 (13.73)</td>
</tr>
<tr>
<td>N-glycosidic bond</td>
<td>28.77</td>
</tr>
</tbody>
</table>

ΔE_{TS} = E(Transition state) – E(Radical anion); using PCM model with ε = 78.39.

Products of the C–O σ and N-glycosidic bond breaking

Both C3–Oy and C5–Oy σ bond ruptures lead to the energetically stable complexes consisting of a phosphate anion and a corresponding carbon-centered neutral radical (Figure 3). In the case of the C5–Oy σ bond breaking, the products are 22.0 and 32.9 kcal/mol more stable than 3′,5′-dCDP− and 3′,5′-dTDP−, respectively (Table 4). Meanwhile, the energies of the C3–Oy σ bond-broken products are 42.0 and 43.1 kcal/mol lower than those of the corresponding reactants, 3′,5′-dCDP− and 3′,5′-dTDP−, respectively. The formation of a H-bond between the phosphate groups in the C5–Oy σ bond-broken product of 3′,5′-dTDP− and in the C3–Oy σ bond-broken products of both radical anions accounts for large energy decrease of these C–O σ bond-broken products. It should be noted that the strong H-bond in the C3–Oy σ bond-broken products includes the neutralizing hydrogen of the phosphate.

Activation energies of the N-glycosidic bond breaking

The transition state for N-glycosidic bond breaking of the radical anion has been located and characterized by the elongated C1–N1 atomic distance (1.873 Å for 3′,5′-dCDP and 1.873 Å for 3′,5′-dTDP). This is further confirmed by the existence of a single imaginary vibrational frequency of 576 i/cm for 3′,5′-dTDP− and 500 i/cm for 3′,5′-dCDP− and the corresponding normal mode representing the C1–N1 σ bond breaking (Figures 1 and 2). The activation energy of the C1–N1 glycosidic bond breaking has been predicted to be 26.21 kcal/mol (Table 2; without ZPE) for 3′,5′-dCDP−, ~4.61 kcal/mol higher than that found for the nucleoside model. An important feature in the glycosidic bond breaking structure representing transition state of cytosine is the existence of a strong H-bonding interaction between the proton at the O5 and the N1 atom [the H(O5)···N1 distance is 1.78 Å in dC−]. However, because of the phosphorylation at the O5 position in 3′,5′-dCDP−, this H-bonding pattern is absent in the corresponding transition state. Therefore, this energy barrier increase is not unexpected. Similarly, in spite of the intramolecular H-bonding between the O5 atom and the proton of the 3′-phosphate, the activation energy for the N-glycosidic bond breaking in 3′,5′-dTDP− is also higher than that in the corresponding nucleoside [19.20 kcal/mol versus 18.9 kcal/mol; (13,21)]. This activation energy barrier increase for the N-glycosidic bond dissociation due to the presence of the adjoining phosphate groups corresponds to the recent experimental observation that while the LEE-induced base release percentage amounts to 16.5 in the oligomer TpT, it is reduced to 0.5 in the oligo-nucleotide pTpT (32).

Similar to the discussed C–O σ bond rupture, the solvent effects raise the energy barrier of the N-glycosidic bond breaking. It is 28.77 kcal/mol for 3′,5′-dTDP− in the PCM model-simulated aqueous solutions. This substantial increase in the energy barrier due to the solvent–solute interactions is in accordance with the largely reduced dipole moment of the corresponding transition state (17.7 Debye versus 22.8 Debye for the optimized radical anion, without vibrational excitation) in aqueous solutions. On the other hand, the solvent–solute interactions only slightly increase the activation energy of the N-glycosidic bond rupture in 3′,5′-dCDP− (26.34 kcal/mol). Correspondingly, the dipole moments of the local minimum structure and the transition state of 3′,5′-dCDP− are very similar (17.9 Debye versus 17.0 Debye).
group. Therefore, this strong interaction would not be expected in real DNA single strands.

The energy release during the $N_1$–$C_1$ bond breaking process is less significant as compared to that during the C–O $\sigma$ bond rupture. $N$-glycosidic bond-broken product of cytidine diphosphate ($P_{dGlyco}$) in the gas phase has the total energy almost the same as that of $3',5'$-dCDP$^{*+}$. This bond-ruptured complex contains a dehydrogenated cytosine anion and a phosphate–sugar–phosphate neutral radical in the gas phase. In parallel, the complex formed by the $N$-glycosidic bond breaking of $3',5'$-dTDP$^{*+} (P_{dTglyco})$ is ~7.67 kcal/mol more stable than $3',5'$-dTDP$^{*+}$. This 7.67 kcal/mol energy release suggests that in the gas phase, the dehydrogenated thymine anion is more stable than the dehydrogenated cytosine anion. Such a phenomenon is in accordance with relatively large electron affinity of the nucleobase thymine.

Inclusion of the polar solvent decreases stability of the $N$-glycosidic bond-broken products compared to the radical anions of the corresponding nucleoside-$3',5'$-diphosphates. The total electronic energy of the product of the $N$-glycosidic bond breaking of $3',5'$-dTDP$^{*+}$ ($P_{dTglyco}$) is 11.12 kcal/mol higher than that of $3',5'$-dCDP$^{*+}$. Meanwhile, the PCM model calculations reveal that the total energy of the $N$-glycosidic bond breaking of $3',5'$-dTDP$^{*+}$ ($P_{dTglyco}$) is 11.12 kcal/mol higher than that of $3',5'$-dCDP$^{*+}$. On the other hand, the C–O $\sigma$ bond rupture products in the polar environment are still significantly more stable than the radical anions of the corresponding nucleoside-$3',5'$-diphosphates. The relative energy (relative to the corresponding radical anion) of the $C_3$–$O_3$ bond-broken product of the cytidine complex is $-22.00$ kcal/mol and that of the thymidine complex is $-27.92$ kcal/mol. Less significantly, the relative energy (relative to the corresponding radical anion) of the $C_5$–$O_5$ bond-broken product of the cytidine complex is $-32.89$ kcal/mol and that of the thymidine species amounts to $-27.72$ kcal/mol. In general, solvent effects increase the energy of the bond-broken products of the pyrimidine diphosphate complexes. It should be noted that this result is contrary to the conclusions of the previous study on the guanosine diphosphate. One concludes that due to the charge relocation to the guanine moiety in aqueous solution, solvent effects

**Table 4.** The relative energies of the bond-broken products (kcal/mol)

<table>
<thead>
<tr>
<th>Bond breaking process</th>
<th>$\Delta E$</th>
<th>$\Delta E_{\text{PCM}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$3',5'$-dCDP$^{*+}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_3$–$O_3$ bond</td>
<td>$-22.00$</td>
<td>$-16.99$</td>
</tr>
<tr>
<td>$C_5$–$O_5$ bond</td>
<td>$-41.97$</td>
<td>$-27.92$</td>
</tr>
<tr>
<td>$N$-glycosidic bond</td>
<td>$-0.03$</td>
<td>$11.12$</td>
</tr>
<tr>
<td>$3',5'$-dTDP$^{*+}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_3$–$O_3$ bond</td>
<td>$-32.89$</td>
<td>$-18.98$</td>
</tr>
<tr>
<td>$C_5$–$O_5$ bond</td>
<td>$-43.09$</td>
<td>$-27.72$</td>
</tr>
<tr>
<td>$N$-glycosidic bond</td>
<td>$-7.67$</td>
<td>$6.57$</td>
</tr>
</tbody>
</table>

$\Delta E = E(\text{Bond-broken product}) - E(\text{Radical anion})$.  
$\Delta E_{\text{PCM}} = E(\text{Bond-broken product}) - E(\text{Radical anion});$ using PCM model with $\epsilon = 78.39$
further stabilize the bond-broken products of the guano-
sine complexes (47).

Both in the gas phase and in the presence of the polar-
izable medium, the C₃-O₅ bond breaking process has the
highest driving force among the three bond breaking
pathways considered in this study. The reaction pathway
through the C₃-O₅ bond breaking is the most thermo-
dynamically favorable. Meanwhile, relatively higher
energies of the N-glycosidic bond-broken products
suggest that the pathway through N₁-C₁ bond rupture
is not thermodynamically preferred.

Molecular orbital (MO) analysis

An analysis of the singly occupied molecular orbitals
(SOMO) provides deeper insights into the electron attach-
ment and the bond breaking mechanisms. Figure 4 illus-
trates the distribution of the unpaired electron along the
LEE-induced bond breaking pathways of the nucleotides.
In the gas phase, the characteristics of the SOMOs of
3',5'-dCDP** and 3',5'-dTDP** indicate that the excess
electron is mainly covalent-bonded to the base moiety
(Figure 4).

One of the important outcomes of the previous studies
on the LEE-induced bond dissociation in the pyrimidine
nucleotides is the conclusion that the excess negative
charge is partly located on the bond to be broken (13).
The SOMO of the transition states in Figure 4 exhibits
similar characteristics of the charge-induced bond
dissociation.

Consistent with the previous study on the LEE-induced
C₃-O₅ bond breaking in the 3'-dCMP and 3'-dTMP, the
excess electron transfer from the base moiety to the
anti-bonding orbital of C₃-O₅ bond through the space
can be identified from the SOMO of the corresponding
transition state. This electron transfer mechanism
accounts for the lower activation energy barrier estimated
for the C₃-O₅ bond dissociation.

The anti-bonding orbital characteristics of the C₅-O₅
bond are obvious from the SOMO characteristics of the
transition state of the C₅-O₅ σ bond rupture. Similarly,
the partial occupation of the N-glycosidic anti-bonding
MO and partial occupation of the π* orbital of the base
moiety is shown in the SOMO of the transition state cor-
responding to the N-glycosidic bond rupture.

The SOMOs of the C-O bond-broken products (Figure 5)
confirm that the radical resides on the C₅' of the
2'-deoxyripyrimidine-C5'(HH')-yl-3'-monophosphate in
C₅-O₅ σ bond ruptured product and on the C₃' of the
2'-deoxyripyrimidine-C3'(H)-yl-5'-monophosphate in
C₃-O₅ σ bond-broken product. The characteristics of
the SOMOs of the N-glycosidic bond dissociation
products in gas phase indicate that the radical is located
on the C1' of 2'-deoxyribose-C1'(H)-yl-3',5'-diphosphate.

The solvent effects modify electron distribution. The
main influence of the solvent effects on the distribution
of the excess electron in the radicals is the increase of the
unpaired electron population on the pyrimidine bases. In
aqueous solution, the characteristics of the SOMO of the
transition state of the C₅-O₅ σ bond rupture indicate that
the excess electron is only slightly shifted to the C₅-O₅
anti-bonding orbital (Supplementary Data). This phenom-
enon might be related to the fact that the C₅-O₅ bond
breaking activation energy barrier increases significantly in
the PCM model studies. Meanwhile, the SOMO of the
transition state of the N-glycosidic bond breaking
process in the aqueous solution is affected less compared
to that in the gas phase. This is directly correlated to the
similar activation energy barrier revealed in the aqueous
solution and in the gas-phase calculations.

Reaction pathways of the LEE-induced
DNA single strands

Based on the electronic affinities and the energy profiles
explored in this study, the possible mechanism of the

![Figure 4](http://nar.oxfordjournals.org/) The SOMOs of radical anion of 3',5'-dCDP** and 3',5'-dTDP**, and the corresponding transition states of the C₅-O₅ bond breaking, C₃-O₅ bond breaking and N-glycosidic bond breaking in the gas phase. The typical characteristics of the σ anti-bond orbital and the breaking bond are shown clearly.
LEE-induced single-strand bond breaking around the pyrimidine sites of the DNA single strands is consistent with the previous mechanism that has been proposed based on the pyrimidine monophosphate models (16,18). That is, the incident electrons bind to the pyrimidine bases in DNA oligomers, forming a base-centered radical anion in the nascent stage. This radical anion is electronically stable enough that either the C–O or glycosidic bond breaking process might compete with the electron detachment and yield corresponding radical fragments and anions.

In the gas phase, the glycosidic bond breaking process requires activation energy as high as 19.19 kcal/mol. Therefore, base release should be excluded based on the mechanisms proposed above. The energy barrier for the C3′–O3′ σ bond cleavage process (~6.0 kcal/mol for both cytidine and thymidine) suggests that this reaction pathway is the most favorable compared to the other possible pathways. On the other hand, the relatively low activation energy barrier (~14 kcal/mol) for the C5′–O5′ σ bond cleavage process indicates that this pathway could be possible, especially when the incident electrons have relatively high energy (a few electron volts). However, as the energy of the incident electrons decrease, the possibility of the reactions through the C3′–O3′ σ bond cleavage pathway is expected to decrease. Therefore, the strand breaks caused by the attachment with near-zero energy electrons is dominated by the C3′–O3′ bond cleavage pathway for the isolated nucleotides.

An application of the PCM model to describe solvent effects excludes accounting for proton transfer or charge transfer processes that might exist between solute and solvent. In this sense, solvent effects greatly increase the activation energies of either C–O σ bond cleavage processes or the N-glycosidic bond breaking process. In the solvated condition, the predicted activation energy barriers of 26–28 kcal/mol for the N-glycosidic bond...
...bond cleavage process eliminate possibility of the observable reactions occurring based on this pathway. It is important to note that the activation energy barrier of the C₅–O₅–C₃–O₃ σ bond cleavage process rises to 13.4 kcal/mol in the PCM calculations, which is about 5 kcal/mol lower than that for the C₅–O₅–C₃–O₃ σ bond cleavage process (18.76 kcal/mol). In comparison with the gas phase, the importance of the C₅–O₅–C₃–O₃ σ bond cleavage process (versus the C₃–O₃ σ bond cleavage process) increases under the solvated condition. However, the C₅–O₅–C₃–O₃ σ bond cleavage pathway still dominates the LEE-induced DNA single strands in the presence of the polarizable surroundings. The energy profiles along the reaction pathways depicted in Figures 6 and 7 clearly reveal that the products of the C₅–O₅–C₃–O₃ σ bond cleavage are favored both kinetically and thermodynamically. Nevertheless, we want to emphasize again that since the activation energy barriers predicted in the polarizable surroundings are in general higher than those in the gas phase, the LEE-induced DNA single strands breaking in the polarizable medium should be less important than the corresponding phenomenon in the gas phase.

Comparison with the experimental results

It is important to note that the models used in this study represent the pyrimidine sites within the DNA single strands. An addition of the methyl group at the 5′-phosphate group prevents the intramolecular proton transfer from the 5′-phosphate group to the bases (at the C6 of either cytosine or thymine) during the formation of the base-centered radical anions. In fact, without the methylation of the 5′-phosphate group, it is hard to prevent this intramolecular proton transfer during the geometry optimization of the radical anions.

For cytidine, the experiments of LEE-induced bond breaks of oligonucleotide tetramer GCAT in the thin solid films revealed the ratio of 5:11 for the bond breaks of C₅–O₅ to the bond breaks of C₃–O₃ (at the site of cytidine) induced by the incident electrons with the energy of 15 eV. This ratio decreases to 3:8 (10 eV) and 4:21 (6 eV) as the energy of the incident electrons diminishes (14). Therefore, one should expect that the ratio of the bond breaks of C₅–O₅ against that of C₃–O₃ induced by the near-zero electron attachment will be even smaller. On the other hand, the percentage of the cytosine base release is negligible. This ratio observed in the experiments clearly follows our theoretical sequence of the bond breaking reaction pathways either in the gas phase or in aqueous solutions.

For thymidine, the experiment of LEE-induced bond breaks of oligonucleotide trimer TTT (TpTpT) (29) in the solid films yields the ratio of 2.5:2.9 for the bond breaks of C₅–O₅ to the bond breaks of C₃–O₃ with the relatively high-energy incident electrons (11 eV). This ratio is also qualitatively consistent with the theoretical predictions.

Considering that the oligonucleotide GCAT is in the thin solid film in the experiment (14), the influence of the surroundings in the thin solid film on the LEE-induced DNA damages is greater than that in the gas phase but smaller than accounted by the solvent effects modeled by the PCM model. This consistency between the theoretical prediction and the experimental observation in the reaction pathway ratio provides strong supportive evidence for the base-centered radical anion mechanism of the LEE-induced single-strand bond breaking around the pyrimidine sites of the DNA single strands mentioned above.

CONCLUSIONS

One of the possible mechanisms for the LEE-induced single-strand breaking in DNA might involve the electron's attachment to the pyrimidine DNA bases and the formation of the base-centered radical anions of the nucleotides in the first step (9,16,18,19). Subsequently, these electronically stable radical anions are capable of undergoing either C–O or glycosidic bond breaking, producing the neutral ribose radical fragments and the corresponding phosphoric anions or base anions. The results of the present study, along with the findings of the earlier investigations (13,16,18) indicate that this mechanism is able to...
elucidate the recent experimental observations on the LEE-induced damages in DNA single strands.

The present results reveal that for the pyrimidine di-phosphates in the gas phase, the strand breaks caused by the attachment of near-zero energy electrons is dominated by the C$_3$–O$_y$ σ bond cleavage pathway. The relatively high-activation energy barrier of the C$_5$–O$_y$ σ bond dissociation process suppresses this C$_5$–O$_y$ σ bond rupture pathway. Due to the presence of the adjacent phosphate groups, the high-activation energy barrier for the glycosidic bond breaking suggests that based on the base-centered radical anion mechanism, LEE attachment is unlikely to directly induce the base release at the pyrimidine sites in the DNA single strands.

In the presence of polarizable surroundings, the interactions between the nucleotides and the polarizable medium increase the activation barriers to 13.4 kcal/mol for the C$_3$–O$_y$ bond cleavage and to 18.8 kcal/mol for the C$_5$–O$_y$ bond cleavage. These relatively high-energy barriers ensure either C$_5$–O$_y$ or C$_3$–O$_y$ bond rupture to take place only in a small rate at the pyrimidine sites in DNA single strands. The values of activation energies of these C–O bond cleavages indicate that C$_3$–O$_y$ bond breaking pathway is superior over that of C$_5$–O$_y$. On the other hand, the comparatively high-energy barrier for the N-glycosidic bond rupture indicates that this reaction pathway is the least possible.

The good agreement between the ratios for the bond breaks of C$_5$–O$_y$, C$_3$–O$_y$ and N-glycosidic bonds observed in the experiment of LEE-induced bond breaks of oligonucleotide tetramer CGAT and trimer TTT in the thin solid films and the theoretical sequence of the bond breaking reaction pathways in the PCM-simulated effects of the polarizable surroundings has been found. This consistency between the theoretical predictions and the experimental observation of the reaction pathway ratio provides strong supportive evidences for the base-centered radical anion mechanism of the LEE-induced single-strand bond breaking around the pyrimidine sites of the DNA single strands.

It should be emphasized that the PCM model only accounts for the effects of the polarizable surroundings. However, there are other important factors governing characteristics of solvated species in aqueous solutions such as microsolvation and proton transfer between solvent and solute, which are not accounted for in the PCM calculations. In addition to the effects of the polarizable surroundings (which increase the activation energy barriers for C–O σ and glycosidic bond cleavage), microhydration and proton transfer between water molecules and the radical anions would further stabilize the reactants by reducing the excessive negative charge of the radical anions. Therefore, electron-induced DNA single-strand bond breaking is not expected to occur in aqueous solutions, as concluded in the experimental studies (48).

SUPPLEMENTARY DATA
Supplementary Data are available at NAR Online.

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