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Low-Energy Electron-Induced DNA Damage: Effect of Base Sequence in Oligonucleotide Trimers

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Abstract: DNA damage induced by low-energy electrons (LEEs) has attracted considerable attention in recent years because LEEs represent a large percentage of the total energy deposited by ionizing radiation and because LEEs have been shown to damage DNA components. In this article, we have studied the effect of base sequences in a series of oligonucleotide trimers by the analysis of damage remaining within the nonvolatile condensed phase after LEE irradiation. The model compounds include TXT, where X represents one of the four normal bases of DNA (thymine (T), cytosine (C), adenine (A), and guanine (G)). Using HPLC-UV analysis, several known fragments were quantified from the release of nonmodified nucleobases (T and X) as well as from phosphodiester C−O bond cleavage (pT, pXT, Tp, and TXp). The total damage was estimated by the disappearance of the parent peaks in the chromatogram of nonirradiated and irradiated samples. When trimers were irradiated with LEE (10 eV), the total damage decreased 2-fold in the following order: TTT > TCT > TAT > TGT. The release of nonmodified nuclobases (giving from 17 to 24% of the total products) mainly occurred from the terminal sites of trimers (i.e., T) whereas the release of central nucleobases was minor (C) or not at all detected (A and G). In comparison, the formation of products arising from phosphodiester bond cleavage accounted for 9 to 20% of the total damage and it partitioned to the four possible sites of cleavage present in trimers. This study indicates that the initial LEE capture and subsequent bond breaking within the intermediate anion depend on the sequence and electron affinity of the bases, with the most damage attributed to the most electronegative base, T.

Introduction

The reaction of low-energy electrons (LEEs) with DNA is relevant to radiobiology because the transfer of energy from ionizing radiation to molecules generates large quantities ($\sim 3 \times 10^4$/MeV) of secondary low-energy electrons (energy $E_o <$ 30 eV).[1] There is a paucity of knowledge about the chemistry of anionic and neutral species resulting from the capture of LEEs by DNA components. In recent years, DNA damage induced by electron attachment has been shown to occur with elementary DNA components (i.e., nucleobases, deoxyribose, and phosphate), nucleosides, nucleotides, oligonucleotides, and plasmid DNA.[2,3] LEEs efficiently attach to DNA components where they form transient anions that dissociate into highly reactive neutral and anion radicals from electronically excited states or by dissociative electron attachment (DEA). Below the energy threshold of electronic excitation, only the dissociation of transient anions involving no electronic excitation (i.e., shape resonances) can create reactive species (i.e., a neutral radical and a stable anion). The heterocyclic and aromatic nature of DNA bases with their relatively low energy π^* orbitals allows them to be particularly efficient in excess electron capture.[4] At low energies ($E < 3$ eV), shape resonances cause electron capture in π^* valence molecular orbitals,[5] giving rise to valence anions. At higher energies (i.e., 10 eV), core excited resonances are formed, which can also decay by DEA. Core-excited resonances result from electron capture by the positive electron affinity of an electronic excited state. Hence, they lie below the parent excited state. When they lie above, they are called core-excited shape resonances. Although all of these processes induce DNA damage, the chemical steps leading to the final modification of the DNA remain to be established.

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(1) Pimblott, S. M.; LaVerne, J. A. *Radiat. Phys. Chem.* **2007**, *76*, 1244–1247.
(2) (a) Denifl, S.; Ptasinska, S.; Cingel, M.; Matejcik, S.; Scheier, P.; Mark, T. D. *Chem. Phys. Lett.* **2003**, *377*, 74–80. (b) Zheng, Y.; Cloutier, P.; Hunting, D. J.; Wagner, J. R.; Sanche, L. *J. Am. Chem. Soc.* **2004**, *126*, 1002–1003. (c) Li, Z.; Zheng, Y.; Cloutier, P.; Sanche, L.; Wagner, J. R. *J. Am. Chem. Soc.* **2008**, *130*, 5612–5613. (d) Zheng, Y.; Cloutier, P.; Hunting, D. J.; Sanche, L.; Wagner, J. R. *J. Am. Chem. Soc.* **2005**, *127*, 16592–16598. (e) Huels, M. A.; Hahndorf, I.; Illenberger, E.; Sanche, L. *J. Chem. Phys.* **1998**, *108*, 1309–1312. (f) Denifl, S.; Ptasinska, S.; Hrusak, J.; Scheier, P.; Mark, T. D. *J. Phys. Chem. A* **2004**, *108*, 6562–6569. (g) Abdoul-Carime, H.; Gohlke, S.; Illenberger, E. *Phys. Rev. Lett.* **2004**, *92*, 168103. (h) Ptasinska, S.; Denifl, S.; Scheier, P.; Mark, T. D. *J. Chem. Phys.* **2004**, *120*, 8505–8511.
(3) For a review, see Sanche, L. In *Wiley Series on Reactive Intermediates in Chemistry and Biology: Entitled Radicals in Nucleic Acids*; Greenberg, M., Ed.; John Wiley & Sons: New York, 2009.
(4) Aflatooni, K.; Gallup, G. A.; Burrow, P. D. *J. Phys. Chem. A* **1998**, *102*, 6205–6207.
(5) Simons, J.; Jordan, K. D. *Chem. Rev.* **1987**, *87*, 535–555.

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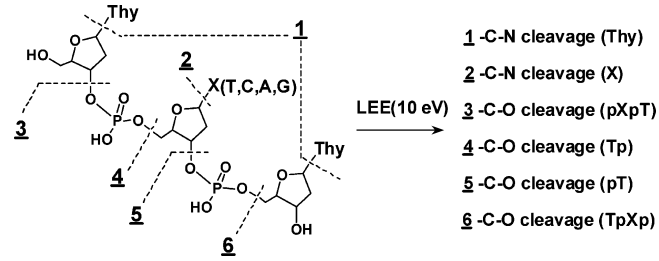
The reaction of LEEs with condensed-phase DNA components has been studied by the technique of electron-stimulated desorption, which allows for the detection of small primary radicals and ions (<100 amu) that undergo desorption from the surface of a solid target upon LEE impact under ultrahigh vacuum (UHV).[3,6] In addition, a number of studies demonstrate the formation of single- and double-strand breaks (SSBs and DSBs) by postirradiation analysis of damaged plasmids using gel electrophoresis.[3,7] Recently, we developed a novel irradiator in which relatively large quantities of biological molecules (10−50 μg) can be bombarded with LEEs.[8] This system provides sufficient degraded material for the preliminary chemical analysis of nonvolatile products. Thus, the present technology allows one to investigate LEE-induced damage of condensed-phase oligonucleotides with subsequent analysis of the residues after irradiation.

Previously, we showed that LEE efficiently induce two types of bond dissociation in model DNA leading to stable nonvolatile products: (1) cleavage of the *N*-glycosidic bond leading to the release of nonmodified nucleobases (e.g., release of thymine from thymidine) and (2) cleavage of the phosphodiester C−O bond leading to the formation of a fragment with an intact terminal phosphate group and unknown modified fragments.[2b,d] The latter cleavage pathway was based on theoretical predictions. Theoretical studies indicate that below about 3 eV, electrons can cleave the C−O bond of the backbone at the 3′ and 5′ positions not only via direct capture by a phosphate group[9] but also via electron transfer.[10] In the latter process, an incoming electron captured by one of the lowest π^*-resonance states of the nucleobase transfers to the sugar−phosphate group of the molecule, where it resides for a sufficiently long time to cause C−O σ-bond rupture. The hypothesis of electron transfer from a base to the phosphate group was first supported experimentally by measurements of SSBs in plasmid DNA in the 0−5 eV range.[11] Later, Zheng et al. bombarded thin molecular films of single-stranded tetramers of DNA with electrons having energies between 4 and 15 eV.[12] They found that both shape and core-excited resonances formed by the initial attachment of the electron to the nucleobase lead to the cleavage of either the *N*-glycosidic or C−O bonds.

Scheme 1. Structure of 5′-TpXpT-3′ and Position(s) of Cleavage for the *N*-Glycosidic Bond (1, 2) and Phosphodiester Bond (3−6)



In the present work, we continue our chemical analyses of nonvolatile damage remaining within LEE-irradiated DNA samples. These studies are complementary to the electron-stimulated desorption of anions and strand-breaking analyses, with the goal of identifying all possible reactions. We focus on the analysis of LEE-induced damage in a series of oligonucleotide trimers by HPLC-UV because the analysis of larger fragments obtained from the bombardment of longer oligonucleotides is complicated by the greater number and lower overall yield of products. The model compounds include TpXpT, where p represents the phosphate unit, X represents one of the four normal nucleobases in DNA, connected to a 2-deoxyribose moiety, and T corresponds to thymine (Scheme 1). Each trimer contains three sites for *N*-glycosidic bond cleavage, resulting in base release (producing T and X), and four sites for phosphodiester bond cleavage (producing pT, pXT, Tp, and TXp), leading to the potential formation of six possible nonmodified fragments.

Experimental Section

Sample Irradiation. Experimental details of the LEE irradiator and the procedure to irradiate samples have been reported elsewhere.[8] Briefly, approximately 80 μg of an HPLC-purified compound was dissolved in 5 mL of nanopure-grade H_2O (Milli-Q water system, 18 MΩ·cm, 25 °C), and the solution was deposited by spin coating onto the inner surface of seven chemically clean tantalum cylinders (3.2 cm × 2.5 cm diameter). The average thickness of the film on the cylinder was 2.5 ± 0.1 nm (four to five monolayers (ML)), assuming that the molecules with a density of 1.7 g cm^{-3}[13] are uniformly distributed on the surface of the cylinder. All manipulations of samples, before and immediately after irradiation, were carried out in a sealed glovebox containing an atmosphere of dry nitrogen. The samples were transferred from the glovebox directly to the LEE irradiation chamber. The latter was subsequently evacuated for ∼24 h to reach a pressure of about 10^{-9} Torr at ambient temperature. The irradiator generated a uniform electron beam over the entire sample surface of the cylinder with an energy resolution of 0.5 eV FWHM. Each cylinder containing the sample was irradiated individually with a constant irradiation time, beam current, and incident electron energy. Under the present conditions, the time of irradiation was 2.5 min, giving a total exposure of approximately 1.0×10^{16} electrons per cylinder. The current and irradiation time were adjusted to give an exposure well within the linear regime of the dose−response curve. Within this linear regime, the film does not accumulate sufficient excess charge to modify the electron energy and induced processes.[2d] The average thickness of the film (2.5 nm) is smaller than both the penetration depth of 10 eV electrons in liquid water or amorphous ice and the inelastic mean free path in biological solids.[14,15]

(6) (a) Aboul-Carime, H.; Cloutier, P.; Sanche, L. *Radiat. Res.* **2001**, *155*, 625–633. (b) Dugal, P.-C.; Abdoul-Carime, H.; Sanche, L. *J. Phys. Chem. B* **2000**, *104*, 5610–5617. (c) Huels, M. A.; Boudaiffa, B.; Cloutier, P.; Hunting, D.; Sanche, L. *J. Am. Chem. Soc.* **2003**, *125*, 4467–4477. (d) Antic, D.; Parenteau, L.; Sanche, L. *J. Phys. Chem. B* **2002**, *104*, 4711–4716. (e) Pan, X.; Cloutier, P.; Hunting, D.; Sanche, L. *Phys. Rev. Lett.* **2003**, *90*, 208102.
(7) (a) Boudaiffa, B.; Cloutier, P.; Hunting, D.; Huels, M. A.; Sanche, L. *Science* **2000**, *287*, 1658–1659. (b) Boudaiffa, B.; Hunting, D.; Cloutier, P.; Huels, M. A.; Sanche, L. *Int. J. Radiat. Biol.* **2000**, *76*, 1209–1221. (c) Cai, Z.; Cloutier, P.; Hunting, D.; Sanche, L. *J. Phys. Chem. B* **2005**, *109*, 4796–4800. (d) Cai, Z.; Cloutier, P.; Hunting, D.; Sanche, L. *Radiat. Res.* **2006**, *165*, 365–371. (e) Panajotovic, R.; Martin, F.; Cloutier, P.; Hunting, D.; Sanche, L. *Radiat. Res.* **2006**, *165*, 452–459.
(8) Zheng, Y.; Cloutier, P.; Wagner, J. R.; Sanche, L. *Rev. Sci. Instrum.* **2004**, *75*, 4534–4540.
(9) Li, X.; Sevilla, M. D.; Sanche, L. *J. Am. Chem. Soc.* **2003**, *125*, 13668–13669.
(10) (a) Barrios, R.; Skurski, P.; Simons, J. *J. Phys. Chem. B* **2002**, *106*, 7991–7994. (b) Berdys, J.; Anusiewicz, I.; Skurski, P.; Simons, J. *J. Am. Chem. Soc.* **2004**, *126*, 6441–6447. (c) Berdys, J.; Skurski, P.; Simons, J. *J. Phys. Chem. B* **2004**, *108*, 5800–5805. (d) Berdys, J.; Anusiewicz, I.; Skurski, P.; Simons, J. *J. Phys. Chem. A* **2004**, *108*, 2999–3005.
(11) Martin, F.; Burrow, P. D.; Cai, Z.; Cloutier, P.; Hunting, D. J.; Sanche, L. *Phys. Rev. Lett.* **2004**, *93*, 068101.
(12) Zheng, Y.; Cloutier, P.; Hunting, D. J.; Wagner, J. R.; Sanche, L. *J. Chem. Phys.* **2006**, *124*, 064710.
(13) Fasman, G. D. *Handbook of Biochemistry and Molecular Biology*, 3rd ed.; CRC Press:: Boca Raton, FL, 1995.
(14) Meesungnoen, J.; Jay-Gerin, J.-P.; Filali-Mouhim, A.; Mankhetkorn, S. *Radiat. Res.* **2002**, *158*, 657–660.

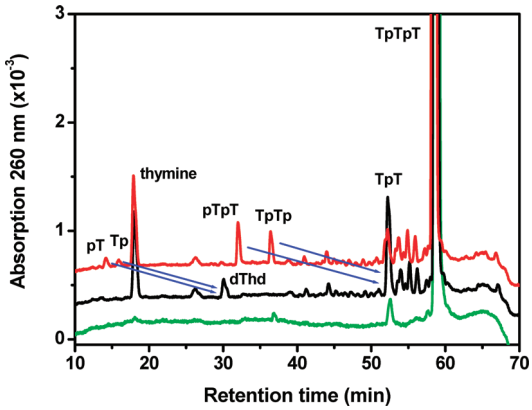


Figure 1. Analysis of TTT (TpTpT) radiation products by HPLC-UV detected at 260 nm. The trimer was exposed to 10^{16} electrons with an energy of 10 eV. The lower chromatogram (green) depicts the analysis of a nonirradiated sample. The upper and middle chromatograms show the corresponding irradiated sample, which was divided into two parts: one was treated with alkaline phosphatase (middle; black) and the other received no treatment (upper; red). Blue arrows illustrate the conversion of products with a terminal phosphate to that without.

Therefore, one can expect that electrons impinging on the film undergo single inelastic scattering events with the molecules.

HPLC Analysis. After irradiation with LEE, the samples were transferred from ultrahigh vacuum to the dry-nitrogen-purged glovebox. The compounds and their radiation products were recovered from the surface of tantalum cylinders by the addition of 12 mL of nanopure-grade H_2O (Milli-Q water system, 18 MΩ·cm, 25 °C). The nonirradiated samples (three cylinders) and the irradiated samples (four cylinders) were pooled, frozen, and lyophilized to dryness. The nonirradiated and irradiated samples were dissolved in 150 and 200 μL of nanopure grade H_2O, respectively, in order to have an equal amount of product per volume in each sample. Half of the sample was analyzed by HPLC. The other half was first treated with alkaline phosphatase (1 unit, Roche Applied Science) for 1 h at 37 °C to remove the terminal phosphate group of nucleotides and then analyzed by HPLC under the same conditions as for the nontreated sample. In the chromatogram, the identity of DNA fragments containing a terminal phosphate group was supported by their conversion to derivatives without a terminal phosphate group upon treating with alkaline phosphatase (Figure 1). The HPLC system consisted of a Waters alliance HT system (model 2795) equipped with a refrigerated autosampler and a dual-wavelength absorbance detector (model 2487). The separation of products was achieved using an analytical YMC-Pack ODS-A column (250 × 6 mm^2) maintained at 30 °C, using a linear gradient from 1 to 10% acetonitrile in buffer containing 25 mM NaH_2PO_4 (pH 5.7) over an interval of 60 min and at a flow rate of 1.0 mL/min. All products were detected at 210 and 260 nm. The yield of damage in terms of the number of damaged molecules per 1000 target molecules was estimated by comparing the peak for the nonmodified trimer in the chromatograms of irradiated and nonirradiated samples. The yield of LEE-induced products was determined by calibration with authentic reference compounds.

Reference Compounds. Thymine (T), cytosine (C), adenine (A), guanine (G), thymidine (dThd), and mononucleotides (pT and Tp) were purchased from Sigma-Aldrich (St. Louis, MO). In addition, several standard dinucleotide fragments of TpXpT containing either a 3′ or 5′ terminal phosphate group were prepared by the enzymatic digestion of the corresponding trimers with micrococcal nuclease (Roche Applied Science, giving TXp) and with P1 nuclease (MP Biomedical, giving pXT).[16] TpXpT was purchased from Alpha DNA (Montreal, QC) and UCDNA Services (Calgary, AB). All samples were purified by HPLC-UV using the same methods as described above for irradiated samples except that a volatile buffer solution (i.e., triethylammonium acetate (20 mM, pH 7)) was used. The purified solution was lyophilized to dryness and redissolved in nanopure-grade H_2O before spin-coating and irradiation. The yield of LEE-induced DNA fragments was determined by comparison of the peak area at 260 nm with the peak area of standard solutions prepared from commercially available compounds (nucleobases, 2′-deoxynucleosides, and mononucleotides (pT and Tp)). In addition, other fragments containing a terminal phosphate (pXpT and TpXp) were obtained by the partial digestion of oligonucleotide trimers with nucleases together with HPLC purification of the fragments. The concentration of standard solutions of trimers and their fragments was estimated by their optical absorption at 260 nm taking the given molar absorptivity of DNA bases (http://www.basic.northwestern.edu/biotools/oligocalc.html). The optical density was measured using a spectrophotometer (Hitachi U-2000).

Results and Discussion

This work focuses on LEE-induced damage that arises from the cleavage of the *N*-glycosidic bond and phosphodiester bonds of oligonucleotide trimers containing T at the terminal positions and different DNA bases at the central position. The analysis of damage was limited to nonmodified fragments of TXT, including nucleobases, mononucleotides, and dinucleotides. After the irradiation of trimers with 10 eV electrons, six known fragments were identified and quantified by HPLC-UV (Figures 1 and 1S-3S; Table 1). These results, as well as those from previous studies,[2] indicate that small DNA components and oligonucleotides undergo two major reactions with LEEs (Scheme 1): cleavage of the C−N bond to give nonmodified nucleobases (i.e., base release of T or X from TXT) and cleavage of the C−O phosphodiester bond to give nonmodified fragments with a terminal phosphate (i.e., pT, Tp, pXT, and TXp from TTT). The remaining products include modified trimers and their fragments, which have not yet been identified in our studies. Previously, we reported a higher amount of total damage for TTT in a series of experiments with small DNA components containing T (155 (previous work) instead of 92 (this work) per 1000 initial molecules).[2c] The difference may be attributed to variations in the uniformity of the film and electron density along the cylinder, which are set for a given series of experiments and are difficult to control between one series of experiments and another. In other words, the error in the values of the total number of degradation products is large from one set of experiments to another but within a given set the error in the relative values is small.

To help interpret our results, we propose a model in which the initial interactions of very low energy electrons (<3 eV) are considered to be similar to those of higher-energy (3−15 eV) electrons (Scheme 2). This model illustrates the pathways leading to base release and C−O bond cleavage following initial electron capture by the nucleobase. In the case of our experiments (i.e., E_o = 10 eV), the incoming electron first forms either a core-excited or a core-excited shape resonance on the nucleobase. The transient anion can decay into three channels: (1) the elastic channel on the left where the electron is re-emitted with the same energy (E_o); (2) the direct DEA channel leading to fragmentation of the parent nucleobase or base release; and (3) the electronically inelastic channel, which can lead to the

(15) (a) Leclerc, G.; Goulet, T.; Cloutier, P.; Jay-Gerin, J.-P.; Sanche, L. *J. Phys. Chem.* **1987**, *91*, 4999–5001. (b) Bass, A. D.; Sanche, L. *Radiat. Environ. Biophys.* **1998**, *37*, 243–257.

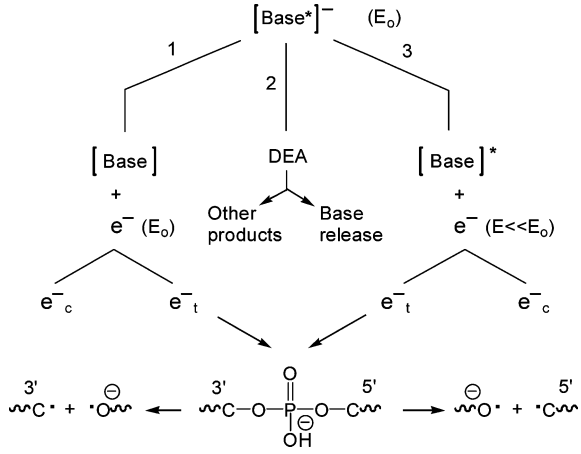
(16) White, J. S. *Source Book of Enzymes*; CRC Press: Boca Raton, FL, 1997.

Table 1. Yield of Products from LEE-Induced DNA Damage

sample[a]		base release				phosphodiester bond cleavage						
TXT	damage[b]	thymine	X	sum	%[c]	pT	Tp	pXT	TXp	sum	%[d]	ratio[e]
X = thymine	92	17.0		17.0	18.5	1.6	1.2	4.3	2.7	9.8	10.7	1.73
X = cytosine	76	12.3	0.4	12.7	16.7	1.5	1.0	2.5	2.1	7.1	9.3	1.79
X = adenine	56	10.7	n.d[f]	10.7	19.1	1.4	1.2	2.0	2.0	6.6	11.8	1.62
X = guanine	37	8.9	n.d[f]	8.9	24.1	1.8[g]	1.4[g]	2.1	1.9	7.2	19.5	1.24

[a] DNA samples and fragments written from 5′ to 3′ with p indicating the position of terminal phosphate groups. [b] Total damage includes all losses of initial targeted molecules based on HPLC-UV analysis. The values are expressed as the ratio of damaged molecules to 1000 initial target molecules and are the average of three independent experiments; SD = ±20%. [c] Percentage of C−N cleavage based on the total damage. [d] Percentage of C−O cleavage based on the total damage. [e] Ratio of C−N to C−O cleavage. [f] Not detected. [g] The sum of pT and Tp is significantly different for TGT compared to that of the other trimers (independent *t* test $P < 0.05$).

Scheme 2. Decay Channels of Transient Negative Ions of DNA Bases Formed at an Initial Electron Energy of E_o[a]



[a] Pathways 1−3 represent the elastic ($E = E_o$), dissociative electron attachment (DEA), and electronically inelastic ($E \ll E_o$) channels, respectively. e_c^- represents the electron re-emitted into the continuum. e_t^- represents the transfer of an electron from the base to the phosphate unit. The transient anion formed on the phosphate unit dissociates, leading to C−O bond cleavage.

electronic excitation of a base and the release of a very low energy electron ($E = 0-3$ eV). In cases 1 and 3, the electron can be re-emitted into the continuum (e_c^-) or transfer (e_t^-) within DNA. When the extra electron transfers to and localizes on the sugar−phosphate group, the C−O σ bond breaks via DEA. According to the previously mentioned investigations and recent theoretical calculation on electron diffraction,[17] it is reasonable to assume that breakage of the C−O bond within DNA occurs principally via pathway 3 for $E_o = 10$ eV. Thus, LEE-induced damage near 10 eV can be discussed in terms of theoretical models for electrons of lower energies (0−3 eV).

Sensitivity of DNA Bases to LEE-Induced Damage. The introduction of cytosine, adenine, and guanine at the central position of oligonucleotide trimers resulted in a considerable decrease in total damage as estimated by HPLC-UV analysis (Table 1). This corresponds to about a 2.5-fold decrease in total damage as a result of changing the central thymine moiety to a guanine moiety. Thus, the sensitivity of DNA bases to LEEs decreases in the following order: T > C > A > G. Interestingly, the trend correlates with the electron affinity of DNA bases, as determined experimentally[18] and as supported by theoretical calculations.[19,20] Seidel et al. measured the reduction potentials (V vs NHE) of the nucleosides in acetonitrile (AN) and *N*, *N*-dimethylformamide (DMF) solution. The results showed that the experimental reduction potentials of the 2′-deoxyribonucleosides follow the order of dThd (−2.18 V) > dCyd (−2.35 V) > dAdo (−2.52 V) > dGuo (<−2.76 V), indicating that dThyd is the easiest 2′-deoxyribonucleoside to reduce and dGuo is the hardest 2′-deoxyribonucleoside to reduce.[18] In other words, near zero eV electrons will preferentially flow to dThd. Using DFT calculations, Richardson et al. predicted that the electron affinities of 2′-deoxyribonucleosides also followed the same order with dThd (0.44 eV) > dCyd (0.33 eV) > dGuo (0.09 eV) ≈ dAdo (0.06 eV).[19] Li et al. calculated the electron affinities of different nucleobases by using DFT methods, and they predicted the same order of electron affinity for nucleic acid bases (T > C > A > G).[20] Our results suggest that LEE, which loses most of its energy via pathway 3 in Scheme 2, may preferentially cause damage to pyrimidine bases in DNA. Similarly, the same trend is observed for solvated electrons such that T and C are the most susceptible DNA bases.[21] The effect of electron affinity on LEE-induced damage is likely related to the formation of initial transient negative ions and electron transfer between DNA bases and from the base to the phosphate group (as discussed below).

Interaction of LEE with DNA Base Arrays (Trimers). The total damage decreases from 9.2 to 3.7% of the total number of bombarded molecules when the middle DNA base of TTT is substituted with alternative nucleobases (Table 1). From a classical point of view, these results are surprising. Even if we assume that the replaced base does not participate any more in the damage, the maximum reduction of damage should be a third of the original damage, giving 7.4% damaged molecules instead of 3.7%. These results suggest that the initial interaction of LEEs or the subsequent chemistry of bases in a trimeric configuration is greatly influenced by the presence of neighboring bases. This effect may be related to the electron affinity of the bases as a determining factor in the capture of LEEs. For example, the electron affinity of trimeric models of XBZ has been examined through theoretical calculations[22,23] indicating that the electron affinity of DNA bases (B in XBZ) is greatly influenced by the adjacent bases. When X and Z are pyrimidine bases, the trimeric XBZ anion is adiabatically stabilized such

(17) (a) Caron, L. G.; Sanche, L. *Phys. Rev. A* **2005**, *72*, 032726. (b) Caron, L. G.; Sanche, L. *Phys. Rev. A* **2004**, *70*, 032719. (c) Caron, L. G.; Sanche, L. *Phys. Rev. Lett.* **2003**, *91*, 113201.
(18) Seidel, C. A. M.; Schulz, A.; Sauer, M. H. M. *J. Phys. Chem.* **1996**, *100*, 5541–5553.
(19) Richardson, N. A.; Gu, J.; Wang, S.; Xie, Y.; Schaefer, H. F. *J. Am. Chem. Soc.* **2004**, *126*, 4404–4411.
(20) Li, X.; Cai, Z.; Sevilla, M. D. *J. Phys. Chem. A* **2002**, *106*, 1596–1603.
(21) von Sonntag, C. *The Chemical Basis of Radiation Biology*; Taylor and Francis: London, 1987.
(22) Kobytecka, M.; Leszczynski, J.; Rak, J. *J. Am. Chem. Soc.* **2008**, *130*, 15683–15687.
(23) Voityuk, A. A.; Michel-Beyerle, M.-E.; Rösch, N. *Chem. Phys. Lett.* **2001**, *342*, 231–238.

that the electron affinity of B increases. In contrast, when X and Z are purine bases, the trimeric XBZ anion becomes adiabatically unstable, leading to a decrease in the electron affinity of B.

The ability of trimers to develop intramolecular base stacking or form aggregates upon evaporation into the condensed phase may lead to changes in the energy levels of trimers and the probability of electron transfer between the bases. In particular, base stacking allows the orbitals of neighboring bases to overlap and form novel ground to excited-state transitions, which may affect the initial formation of transient ions from LEE attachment. Furthermore, one expects an overlap of the extra electron wave function of DNA bases in oligonucleotide trimers. This overlap is likely to be larger in the homotrinucleotides, providing a maximum exchange of captured electrons between bases. If the wave functions are coherent at the resonance energies or at lower energies in pathway 3 (Scheme 2), then they can considerably increase the electron capture cross section of the entire oligonucleotide. Replacing T with another base in the trimer would tend to break this coherence and reduce the electron-capture probability and possibly the electron-transfer probability in pathway 3. In fact, in recent model calculations[17a,b] it has been shown that, because its wavelength is longer than the interunit distances in DNA at low energies ($E_o \leq 3$ eV), the electron scattered within DNA has a high probability of being delocalized in the molecule. Thus, in the case of pathway 3, the inelastically scattered electron is first likely to undergo multiple intersite transfers before being captured at the phosphate unit in a resonant state. As shown theoretically, the partial wave content of the electron wave function inside DNA then becomes dominated by constructive interference below 6 eV; this condition considerably enhances (i.e., up to at least an order of magnitude) the transfer probability.[17a,b] It is therefore highly probable that there exists in TTT a strong coherence enhancement of the electron wave function owing to the homogeneity and periodicity in the positions of the T bases. When this periodicity and coherence of the extra electron wave function are broken in TXT, a reduction in electron transfer will occur with a corresponding reduction in C−O bond cleavage and base release. On the basis of these considerations, for nearly thermalized electrons (pathway 3), the exchange of electrons between the bases is expected to be proportional to the electron affinity of the middle base. The exchange of such electrons would therefore be in the order of TTT > TCT > TAT > TGT, which corresponds to the order of decreasing DNA damage observed experimentally.

Base Release at the Terminal Positions. The reactions of LEE with oligonucleotide trimers led to the release of nonmodified nucleobases with a strong bias for the release of thymine from the terminal positions of TXT (17−24% of the total damage). This result is also supported by the inefficient release of bases from the central position of mixed trimers in comparison to the release of thymine from terminal positions. For example, the release of cytosine was 15-fold less than the release of thymine from TCT (0.4 compared to 6.15 (12.3/2) molecules per initial target molecule; Table 1). The release of purine bases from the central position (i.e., TAT and TGT) could not even be detected in our analyses, indicating that it is even less than that of cytosine. These results may simply reflect the stronger attraction of the extra electron by the deeper potential of T when compared to that of the other bases. However, even in TTT, *N*-glycosidic bond cleavage at the terminal bases is much larger. This phenomenon was also observed in former studies with smaller fragments (T and TT) and oligonucleotide tetramers.[2b–d] Thus, it appears that when LEEs are initially captured by the trimer they efficiently localize at the termini to induce C−N bond cleavage and the release of nonmodified thymine. It is also possible that when the electron is located on the middle base, electron transfer via pathway 3 is favored as deduced previously by the analysis of LEE-induced products from TTT.[2c] In fact, both processes could act synchronously to reduce C−N bond cleavage in the middle.

In theoretical studies of base release from nucleosides, Li et al. predicted that the activation barrier for the C−N bond-breaking process was about 20 kcal/mol and Gu et al. predicted 18.9−21.6 kcal/mol (gas phase).[24,25] In contrast, a higher-energy barrier for C−N bond cleavage was predicted for the central sugar−cytosine C−N bond of the CCC trimer (43 kcal/mol using very low energy electrons (0.8 eV); calculations made in a gas-phase single-DNA-strand trimer).[26] The higher-energy barrier for C−N bond breaking at the central position may also be related to the lack of C−N bond cleavage at this position.

Effect of G on C−O Bond Cleavage. The introduction of cytosine, adenine, and guanine into the central position of oligonucleotide trimers in general resulted in minor changes in the yield of products arising from C−O bond cleavage (i.e., the release of monomers and dinucleotides from trimers (Table 1)). There was a significant increase in the release of pXT and TXp fragments that resulted from C−O cleavage of the sugar moiety connected to the terminal base; however, this can probably be attributed to enhanced electron localization at the termini as described previously. Interestingly though, there was a significant increase in the yield of pT and Tp fragments arising from C−O cleavage at the internal position for TGT in comparison to that for the other trimers (Table 1). Moreover, the total damage decreased 2-fold in going from TTT to TGT, and thus the presence of guanine in comparison to other bases significantly increased the C−O bond cleavage as a percentage of the total damage. Thus, C−O bond cleavage at positions 4 and 5 of oligonucleotide trimers (Scheme 1) is favored when the central nucleobase is guanine.

The finding that guanine enhances C−O bond cleavage is supported by theoretical studies as related to differences in electron density. In particular, Gu et al. recently showed that 2′-deoxyadenosine-3′,5′-diphosphate (pAp) and 2′-deoxyguanosine-3′,5′-diphosphate (pGp) radical anions formed below 3 eV (i.e., via pathway 3 in Scheme 2 in our case) have low vertical detachment energies and the excess electron, attached to pGp, exclusively resides in the vicinity of the 3′-phosphate group of the nucleotide containing guanine when it is solvated.[27] The high excess electron density on the phosphate group of pGp might result from electron transfer from the guanine moiety to induce 3′ C−O bond cleavage giving pT as the product. Ray et al. also suggest experimentally that guanine, because of its high dipole moment, could function as a gateway when capturing LEEs and that when the electron is captured by the base it rapidly transfers to the DNA backbone.[28] In contrast, Schyman et al. do not observe the excess electron near the phosphate

(24) Li, X.; Sanche, L.; Sevilla, M. D. *Radiat. Res.* **2006**, *165*, 721–729.
(25) Gu, J.; Xie, Y.; Schaefer, H. F. *J. Am. Chem. Soc.* **2005**, *127*, 1053–1057.
(26) Anusiewicz, I.; Berdys, J.; Sobczyk, M.; Skurski, P.; Simons, J. *J. Phys. Chem. A* **2004**, *108*, 11381–11387.
(27) Gu, J.; Xie, Y.; Schaefer, H. F. *Nucleic Acids Res.* **2007**, *35*, 5165–5172.
(28) Ray, S. G.; Daube, S. S.; Naaman, R. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 15–19.

Table 2. Different Calculations for the Energy Barrier of C−O Bond Rupture after LEE Attachment

			ΔE^a		
authors	model	method/basis sets	gas phase	aqueous solution[b]	reference
Schyman et al.	Gp	B3LYP/DZP++	10.28	5.25	29
Gu et al.	Tp	B3LYP/DZP++	7.06	13.73	30a
Gu et al.	Cp	B3LYP/DZP++	6.17	12.82	30a
Bao et al.	pT	B3LYP/DZP++	13.84	17.86	30a,b
Bao et al.	pC	B3LYP/DZP++	14.27	17.97	30a,b
Sevilla et al.	pT	B3LYP/6-31G*	14.8	28.9	31
Simons et al.	Tp	SCF/6-31+G*	8.26−13.01[c]	6.71−24.53[c]	10c, 26
Simons et al.	Cp	SCF/6-31+G*	8.38−15.6[d]	5.1−28.1[d]	10d, 26

[a] ΔE = Energy barrier for 3′C−O or 5′C−O bond breaking (in kcal/mol). [b] Polarizable continuum model (PCM) using water as a solvent with the dielectric constant of $\varepsilon = 78.39$. [c] Electron energy E (eV) between 0.25 and 1.0. [d] Electron energy E (eV) between 0.2 and 1.5.

group in an aqueous solution in their theoretical study of LEE attachment to guanosine 3′-monophosphate, but they obtain a low activation energy for the phosphodiester bond rupture induced by LEEs in both the gas phase and aqueous solution.[29] For LEE attachment, they predicted an energy barrier of 10.3 kcal/mol for the 3′ C−O bond breaking in gas phase and 5.3 kcal/mol in aqueous solution. The energy of 3′ C−O bond breaking for G derivatives was generally lower than that for thymine and cytosine derivatives. (The values obtained from different calculations are summarized in Table 2.) From these and our results, one can expect that DNA strand breaks via pathway 3 will most likely occur when a LEE is initially attached to guanine.[10c,d,29–31] In fact, Solomun et al. recently reported a linear increase in DNA fragmentation as a function of the number of guanine residues upon exposure of self-assembled oligonucleotide monolayers to 1 eV electrons.[32]

Summary

We investigated damage induced by 10 eV electrons on TXT where X = T, C, A, or G. The total damage decreases in the order of T > C > A > G. Considering that base release occurs almost exclusively from the termini, we conjecture that in a long DNA polymer, base release will be suppressed (because of the lack of termini) and C−O cleavage will be favored with respect to total damage. The observed dependence of the yield of various products on the nature of the central base can be explained by the influence of base electronegativity on either initial electron capture or electron transfer between the bases. Constructive interference of the wave function of electron capture by the trimer would favor transient anion formation in TTT whereas substitution by a different base in the middle would have a tendency to destroy this coherence and hence lower the capture probability. Furthermore, when the electron is temporarily located on a base, the transient anion thus formed could transfer within its lifetime to other bases and the backbone. Here again, the nature of the base and the secondary structure of the base combination are expected to play a major role. Indeed, the sensitivity of T compared to that of other bases suggests that sequences containing consecutive pyrimidines are probably preferential sites for LEE-induced DNA damage.[23] Paradoxically, though, the pathway leading to C−O bond cleavage is enhanced when the C−O bond is next to G in comparison to other bases.

Supporting Information Available: Analysis of LEE-induced damage of TCT, TAT, and TGT by HPLC-UV. This material is available free of charge via the Internet at http://pubs.acs.org.

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(29) Schyman, P.; Laaksonen, A. *J. Am. Chem. Soc.* **2008**, *130*, 12254–12255.
(30) (a) Gu, J.; Wang, J.; Leszczynski, J. *J. Am. Chem. Soc.* **2006**, *128*, 9322–9323. (b) Bao, X.; Wang, J.; Gu, J.; Leszczynski, J. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 5658–5663.
(31) Kumar, A.; Sevilla, M. D. *J. Phys. Chem. B* **2007**, *111*, 5464–5474.
(32) Solomun, T.; Seitz, H.; Sturm, H. *J. Phys. Chem. B* **2009**, *113*, 11557–11559.