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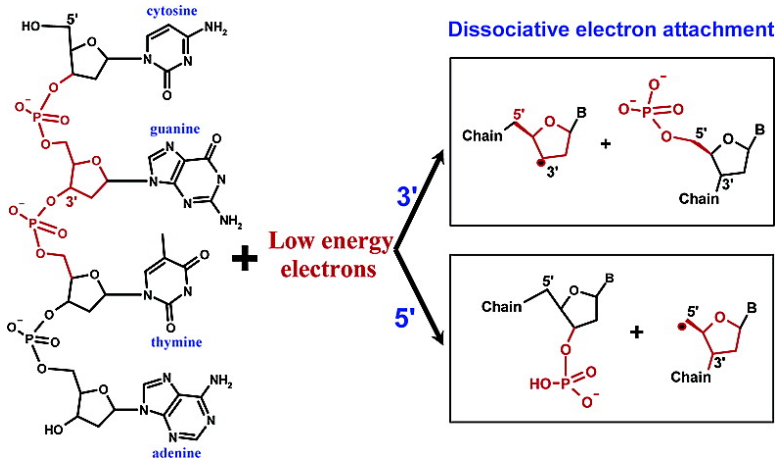

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Chemical Basis of DNA Sugar−Phosphate Cleavage by Low-Energy Electrons

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Abstract: DNA damage by low-energy electrons (LEE) was examined using a novel system in which thin solid films of oligonucleotide tetramers (CGTA and GCAT) were irradiated with monoenergetic electrons (10 eV) under ultrahigh vacuum. The products of irradiation were examined by HPLC. These analyses permitted the quantitation of 16 nonmodified nucleobase, nucleoside, and nucleotide fragments of each tetramer resulting from the cleavage of phosphodiester and *N*-glycosidic bonds. The distribution of nonmodified products suggests a mechanism of damage involving initial electron attachment to nucleobase moieties, followed by electron transfer to the sugar−phosphate backbone, and subsequent dissociation of the phosphodiester bond. Moreover, virtually all the nonmodified fragments contained a terminal phosphate group at the site of cleavage. These results demonstrate that the phosphodiester bond breaks by a distinct pathway in which the negative charge localizes on the phosphodiester bond giving rise to nonmodified fragments with an intact phosphate group. Conversely, the radical must localize on the sugar moiety to give as yet unidentified modifications. In summary, the reaction of LEE with simple tetramers involved dissociative electron attachment leading to phosphodiester bond cleavage and the formation of nonmodified fragments.

Intoduction

DNA is the most important biological target of ionizing radiation.[1] DNA damage induced by ionizing radiation can primarily be attributed to water radiolysis products (hydroxyl radicals, solvated electrons, and H-atoms), radical ion pairs resulting from the ionization of DNA components, and finally, secondary low-energy electrons (LEE). Previously, we demonstrated that direct bombardment of DNA with LEE gives single-strand and double-strand breaks in supercoiled plasmid DNA.[2,3] The ability of LEE to damage DNA is supported by extensive and detailed investigations of the basic interactions of LEE with nucleobases,[4−8] ribose derivatives,[9,10] oligonucleotides,[11,12] and plasmid DNA.[13−15] Together, these studies suggest that LEE (0−20 eV) efficiently induce the formation of stable anions and radical fragments within DNA by a resonance process, i.e., dissociative electron attachment (DEA). Although this process likely leads to bond dissociation, the structure of stable products and their mechanism of formation remain to be established. Thus, a major goal of our research is to characterize LEE-induced products and link these products to deleterious effects in radiation biology.

The reaction of LEE 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 that undergo desorption from the surface of a solid target upon electron impact under ultrahigh vacuum (UHV), and in many cases, at cryogenic temperatures.[4−11,13,14] A major limitation of this technique is that it does not permit the detection of nonvolatile products that remain on the target surface. In addition, this technique does not permit the detection of anions or cations without sufficient kinetic energy to escape the surface nor investigation of the chemical fate of initial radicals or ions, which depends on the phase (solid or liquid), as well as the presence of reactive solutes (oxygen, antioxidants). For these reasons, we have developed a novel irradiator in which relatively large quantities of biological molecules (10−50 μg)

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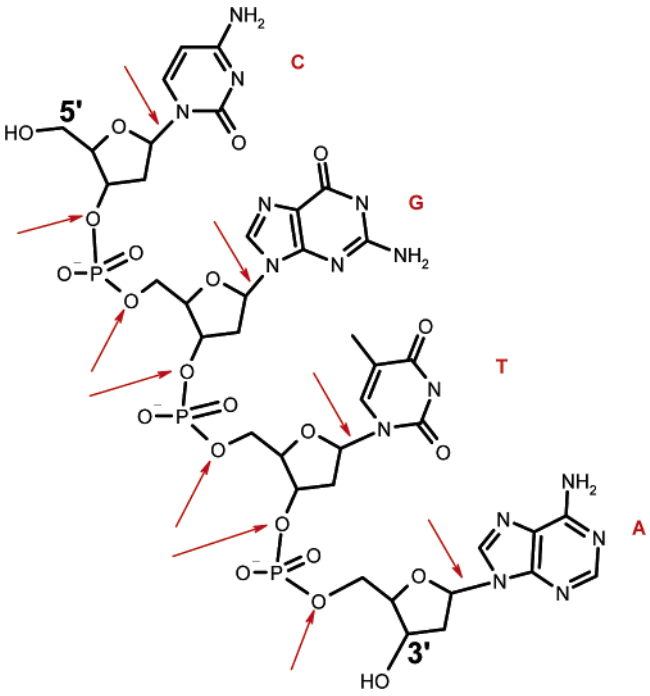
Chart 1. Structure of Oligonucleotide Tetramer CGTA with Sites of Cleavage Marked by Arrows



can be bombarded with LEE at one time.[16] This system provides enough degraded material for preliminary chemical analysis of nonvolatile products. Using this technique, we have demonstrated that LEE efficiently induced *N*-glycosidic bond cleavage of thymidine, liberating nonmodified thymine as a major product.[17a]

The structures of nearly 100 radiation-induced DNA lesions, including isomers, have been characterized from the oxidation of DNA components by hydroxyl radicals and single-electron oxidation as well as from the reduction of DNA components by solvated electrons.[18] One can expect that LEE will produce novel modifications because these electrons have kinetic energy that can induce resonant processes, such as dissociative electron attachment. A good example of the difference between LEE and solvated electrons is that only LEE induce strand breaks in DNA.[19] To continue our work with DNA, we have examined products arising from the irradiation of short oligonucleotides with LEE. The formation of DNA damage was detected by removal of the sample from the irradiated surface followed by HPLC analysis. In the present work, we focused on the analysis of nonmodified fragments of CGTA and GCAT, which arise from cleavage at either the phosphodiester or *N*-glycosidic bonds (Chart 1; sites of cleavage are indicated by arrows). Each tetramer contains six sites for phosphodiester bond cleavage and four sites for *N*-glycosidic bond cleavage, leading to the potential formation of 16 possible nonmodified fragments.

Experimental Section

Sample Irradiation. Experimental details of the LEE irradiator and the procedure to irradiate samples were recently reported.[16] Briefly, 85 nmol of CGTA or GCAT (100 μg) was dissolved in 5 mL of 100% HPLC grade methanol (Fisher Scientific, Montreal, QC), and the solution was deposited by spin-coating onto the inner surface of seven chemically clean tantalum cylinders (12 nmol/cylinder).[16] The average thickness of the film on the cylinder was 2.3 $\pm$ 0.1 nm (5 $\pm$ 0.2 monolayers (ML)), assuming both that the molecules are uniformly distributed on the surface of the cylinder and that the average density of DNA is 1.7 g cm^{-3}.[20] 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 to the LEE irradiation chamber, which was subsequently evacuated for $\sim$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 full width at half-maximum. Each cylinder containing the sample was irradiated individually with possible adjustment of the time of irradiation, beam current, and incident electron energy. Under present conditions, the total electron density transmitted through the sample was approximately 7×10^{14} electrons/cm^2. From 4 to 15 eV, the current and irradiation time were adjusted to give an exposure well within the linear regime of the dose response curve and an equal number of electrons for each sample. The yield of products induced by 15 eV electrons at a constant electron beam flux of 10.6 μA $= 6.6 \times 10^{13}$ electrons/s exhibited a linear relationship as a function of irradiation time up to 6 min. Within this linear regime, we can assume that the film does not accumulate any charge. The average thickness of the film (2.3 nm) was considerably smaller than the penetration depth (5−20 nm) of 4−15 eV electrons in liquid water or amorphous ice.[21a] Because the penetration depth is in fact smaller than the inelastic mean free path for electronic excitation of biological solids (9−28 nm), electrons impinging on the film will be transmitted to the metal substrate and will, at most, lead to single inelastic scattering events with the molecules.[21b]

HPLC Analysis. After irradiation with LEE, the samples were removed from UHV and placed into the dry nitrogen glovebox. The oligonucleotide tetramers and their radiation products were recovered from the surface with 12 mL of degassed 100% HPLC grade methanol (Fisher Scientific), evaporated to dryness by rotary evaporation, and redissolved in 200 μL of Nanopure grade H_2O. The recovery of tetramer from the surface of the cylinder after spin-coating and irradiation in UHV was approximately 70% (8.4 from 12 nmol/cylinder). The low recovery can be attributed to LEE-induced degradation of the tetramer, LEE-induced desorption of molecules from the film surface, and incomplete dissolution of tetramer and products from the tantalum surface. The material recovered from 2 cylinders was combined for subsequent analysis (16.8 nmol/sample). Half of the sample was analyzed by HPLC. The other half was first treated with alkaline phosphatase (AP) (Roche Applied Science) for 1 h at 37 °C to remove the terminal phosphate group of nucleotide-containing fragments and then analyzed by HPLC under the same conditions as the nontreated sample. The HPLC system consisted of a Waters Alliance system equipped with a refrigerated autosampler, a 2690 solvent delivery module, and a 2487 dual wavelength absorbance detector. The mixture of products was separated on an ODS-AQ column (150 $\times$ 6 mm), maintained at 30 °C, using a linear gradient from 1% to 10% acetonitrile in buffer containing 25 mM ammonium acetate (pH 5.7) over an interval of 60 min and at a flow rate of 1.0 mL/min. The analysis of nucleobases (Cyt, Gua, Thy, and Ade) was also carried under the same conditions except with buffer adjusted to pH 5.0. The analysis of mononucleotides

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(Cp, pA, Gp, pT) was also carried out under initially isocratic conditions with 1% acetonitrile. All products were detected at 210 and 260 nm. The peak area for each product was normalized to the area of the parent compound to reduce variations due to the recovery of sample from the cylinder and subsequent reduction of the sample volume by rotary evaporation. The yield of LEE-induced products was determined by calibration with authentic reference compounds. The concentration of reference compounds was estimated by the molar absorptivity of each nucleotide monophosphate component at 260 nm (pG = 12 010; pC = 7050; pA = 15 200; pT = 8400 mol^{-1} cm^{-1}; taken from http://www.basic.northwestern.edu/biotools/oligocalc.html).

Standard Compounds. Nucleobases (Cyt, Gua, Ade, Thy) and nucleosides (dCyd, dGuo, dAdo, dThd) were purchased from Sigma-Aldrich (St. Louis, MO). Oligonucleotide tetramers and their fragments without a terminal phosphate group were purchased from Alpha DNA (Montreal, QC). They were purified by HPLC, and their structure was confirmed by MSMS analysis (Q-Tof2; Waters/Micromass, Manchester, U.K.) by electrospray injection of samples using 50% acetonitrile in water containing 1 mM NaCl at a rate of 0.6 μL/min in positive mode with 2.3 kV applied to the solvent. DNA fragments gave the following molecular ions $(M + Na)^+$: CGTA (m/z = 1172), GCAT (m/z = 1172), CGT (m/z = 882), GTA (m/z = 882), GT (m/z = 594), CG (m/z = 580), TA (m/z = 578) for CGTA (m/z = 1172), and GCA (m/z = 892), CAT (m/z = 866), GC (m/z = 579), CA (m/z = 563), AT (m/z = 578). In addition, several standard dinucleotide and trinucleotide fragments of CGTA and GCAT containing either a 3′ or 5′ terminal phosphate group were prepared by enzymatic digestion of the corresponding tetramer.[22] The preparation of fragments with a 3′ terminal phosphate was carried out by partial digestion of the tetramers with micrococcal nuclease (Roche Applied Science). This treatment gave two fragments from CGTA (CGp and CGTp) as well as two fragments from GCAT (GCp, and GCAp). The standards for Cp and Gp were difficult to prepare using this procedure because of inefficient hydrolysis of the phosphodiester bond between CG or GC. Thus, Cp and Gp were prepared by complete digestion with an excess of enzyme. Conversely, the treatment of the tetramers with P1 nuclease (MP Biomedical) gave fragments containing a terminal 5′-phosphate, i.e., CGTA gave pA, pTA, and pGTA and GCAT gave pCAT, pAT, and pT. The identity of dinucleotide and trinucleotide fragments containing a terminal phosphate group was subsequently confirmed by their transformation into fragments without a terminal phosphate (e.g., pTA → TA), using alkaline phosphatase, followed by cochromatography of the resulting fragments with standard compounds.

Results

This work focuses on the formation of nonmodified fragments of CGTA and GCAT, which included monomeric components (nucleobases, nucleosides, and mononucleotides) and oligonucleotide fragments (dinucleotides and trinucleotides). For electron energies between 4 and 15 eV, the yield of these products increased as a function of energy from 4 eV to attain a maximum at 10−12 eV, and then the yield decreased to a minimum value at 14 eV. The profile of products appeared to be the same for electrons within this range of energies (4−15 eV). The percentage of nonmodified fragments to total decomposition products (~25%) was estimated from the sum of all nonmodified fragments divided by the total peak area of all products that arise from LEE irradiation as measured at 260 nm. Thus, we estimate that nonmodified fragments reported in this work represent about 50% of the total decomposition of tetramer assuming that each nonmodified fragment gave a corresponding modified fragment of the tetramer. The nonmodified fragments of CGTA and GCAT were identified in the product mixture by comparison of their chromatography properties with standard compounds, using the usual conditions of separation (0−20% acetonitrile), and/or alternative conditions with a slower gradient or lower pH to improve the separation of closely eluting products. The identity of fragments containing a terminal phosphate was supported by their conversion to fragments without the phosphate upon treatment with alkaline phosphate. The peak at 20 min in both nonirradiated and irradiated samples varied with the quality and batch of commercially available methanol and, thus, may be attributed to the presence of an impurity. In addition, a number of oligonucleotide products were observed in nonirradiated samples (Figures 1 and 2). These products were absent in purified tetramer, and thus, they must arise from degradation of the tetramer during sample preparation, including contact and removal of tetramer from the Ta surface.[12,17a]

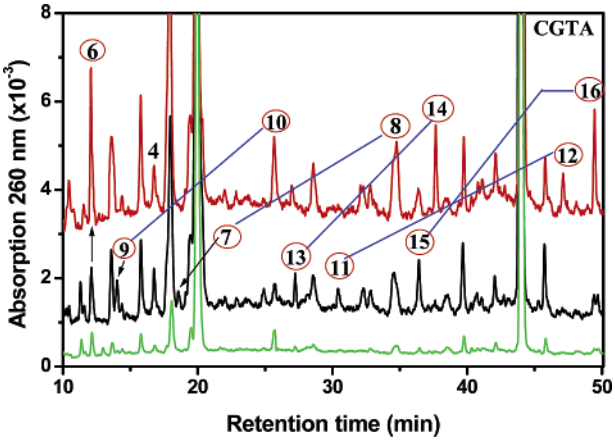

Figure 1. HPLC analysis of LEE-induced damage to CGTA. Tetramer was exposed to 1.9×10^{16} electrons with an energy of 10 eV. The lower chromatogram (green) depicts the analysis of a nonirradiated sample. The upper and middle chromatograms show the corresponding irradiated sample, which was divided in two and treated with alkaline phosphatase (upper; red) or without enzyme (middle; black), respectively. Blue lines illustrate the conversion of products with a terminal phosphate to those without. The identity of the peaks is given in Table 1.

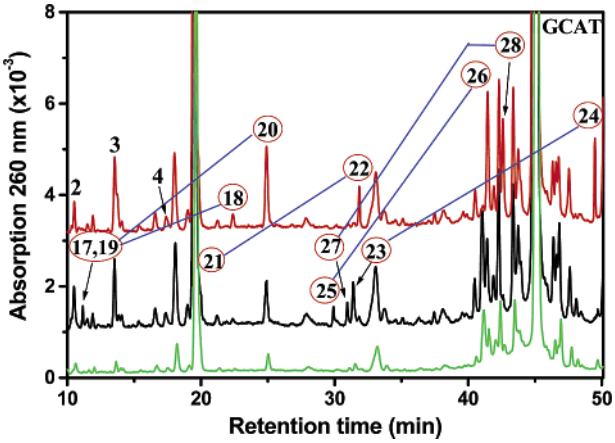

Figure 2. HPLC analysis of LEE-induced damage to GCAT. Tetramer was exposed to 1.9×10^{16} electrons with an energy of 10 eV. The lower chromatogram (green) depicts the analysis of a nonirradiated sample. The upper and middle chromatograms show the corresponding irradiated sample, which was divided in two and treated with alkaline phosphatase (upper; red) or without enzyme (middle; black), respectively. Blue lines illustrate the conversion of products with a terminal phosphate to those without. The identity of the peaks is given in Table 1.

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Table 1. Yield of LEE-Induced Products of Irradiated Tetramers[a]

CGTA (16.8 nmol)			GCAT (16.8 nmol)		
product	peak no.	yield (nmol)	product	peak no.	yield (nmol)
		Nucleobases			
Cyt	1	0.27 ± 0.05	Gua	2	0.22 ± 0.03
Gua	2	n.d[b]	Cyt	1	0.03 ± 0.05
Thy	3	0.12 ± 0.02	Ade	4	0.11 ± 0.01
Ade	4	0.35 ± 0.07	Thy	3	0.35 ± 0.02
		Nucleosides and Mononucleotides			
Cp	5	0.29 ± 0.06	Gp	17	0.11 ± 0.01
dCyd	6	0.06 ± 0.01	dGuo	18	0.00 ± 0.01
pA	7	0.19 ± 0.04	pT	19	0.23 ± 0.01
dAdo	8	0.05 ± 0.01	dThd	20	0.10 ± 0.01
		Dinucleotides and Trinucleotides			
CGp	9	0.19 ± 0.04	GCp	21	0.16 ± 0.01
CG	10	n.d.	GC	22	n.d.
pTA	11	0.11 ± 0.02	pAT	23	0.22 ± 0.01
TA	12	n.d.	AT	24	n.d.
CGTp	13	0.20 ± 0.04	GCAp	25	0.31 ± 0.02
CGT	14	n.d.	GCA	26	0.04 ± 0.01
pGTA	15	0.23 ± 0.05	pCAT	27	0.27 ± 0.01
GTA	16	n.d.	CAT	28	n.d.
total		2.06 ± 0.07			2.15 ± 0.08

[a] Each fragment is written from 5′ to 3′ with p indicating the position of a terminal phosphate group (5′-before or 3′-after DNA base). The yield of products was estimated from the average of three HPLC analyses for CGTA and seven analyses for GCAT, where each analysis was obtained from independent experiments (error = ±SD). The yield of nucleobases (1−4) was estimated from samples treated either with or without alkaline phosphatase. The yield of nucleosides (6, 8, 18, and 20) and fragments not containing a terminal phosphate (10, 12, 14, 16 for CGTA, and 22, 24, 26, 28 for GCAT) were estimated from samples before treatment with enzyme. Fragments containing a terminal phosphate (odd-numbered products from 5 to 15 for CGTA and from 17 to 27 for GCAT) were quantified from the difference in peak area between samples treated with and without enzyme. [b] Not detected.

The reaction of LEE with tetramers led to the release of all four nonmodified nucleobases with a bias for the release of nucleobases from terminal positions. The main nucleobases released from CGTA (Figure 1) included Cyt (peak 1, not shown) and Ade (peak 4), whereas those from GCAT (Figure 2) included Gua (peak 2) and Thy (peak 3). Table 1 gives the amount of nonmodified fragment formed in each sample of 16.8 nmol, based on the analyses of several samples by HPLC. These results show that the release of Thy from the internal position of CGTA was 3-fold less than from the terminal position of GCAT. Similarly, there was a bias for the release of Ade from internal compared to terminal positions. In contrast, the release of Cyt was much more pronounced from the terminal than from the internal position (10-fold). On the other hand, the release of Gua was not at all detected from the internal position of CGTA. The reaction of LEE with tetramers also led to the release of nonmodified nucleosides and mononucleotides. In contrast to nucleobases, the release of nucleosides and nucleotides occurred exclusively from the terminal positions of each tetramer. The reaction of LEE with CGTA gave dCyd (peak 6) and dAdo (peak 8) as nucleoside fragments together with Cp (peak 5, not shown) and pA (peak 7) as mononucleotide fragments (Figure 1; note that dAdo (peak 8) appeared as a sharp peak on the shoulder of a much broader peak in this analysis). On the other hand, the reaction of GCAT gave dThd (peak 20), traces of dGuo (peak 18), as well as the corresponding mononucleotides, pT (peak 19) and Gp (peak 17) (Figure 2). From these results, we conclude that LEE induces the release of nucleoside or mononucleotide fragments from external positions but not from internal positions of the two tetramers. Because the release of monomeric fragments from internal positions requires the cleavage of two phosphodiester bonds, the lack of these fragments in the product mixture indicates that no more than one electron reacts with each target molecules under our conditions.

The reaction of LEE with CGTA and GCAT led to the liberation of several nonmodified oligonucleotide fragments. For each tetramer, there were eight possible dinucleotide and trinucleotide fragments resulting from 3′ or 5′ cleavage of the four internal phosphodiester bonds. In the case of irradiated CGTA, the chromatogram showed the formation of CGp (peak 9), pTA (peak 11), CGTp (peak 13), and pGTA (peak 15) (Figure 1, middle chromatogram). In contrast, the corresponding fragments without a terminal phosphate group were minor or not detected in the initial product mixture (CG (peak 10), TA (peak 12), CGT (peak 14), and GTA (peak 16)). In the case of irradiated GCAT, the major dinucleotide and trinucleotide fragments were also found to contain a terminal phosphate group. The chromatogram showed the formation of fragments with a terminal phosphate group, including pAT (peak 23), GCAp (peak 25), and pCAT (peak 27), whereas the yield of fragments without a terminal phosphate was minor (GCA (peak 26)) or not detected (GC (peak 22), AT (peak 24), CAT (peak 28)) (Figure 2, middle chromatogram). Finally, the same pattern of cleavage was observed for the loss of mononucleotides from terminal positions of tetramers. Although this cleavage gave fragments with and without a terminal phosphate, the yield of fragments with a phosphate was much greater than that without a phosphate. For example, the yield of Cp was 0.29 ± 0.02 whereas that of dCyd was only 0.06 ± 0.01 in irradiated CGTA (Table 1). The same differences were also observed for pA and dAdo in irradiated CGTA as well as Gp and dGuo, and pT and dThd in irradiated GCAT. Thus, the formation of 6 major nonmodified fragments out of a total of 12 possible fragments for each tetramer indicates that LEE induces the cleavage of phosphodiester bonds to give nonmodified fragments with a terminal phosphate rather than a terminal hydroxyl group.

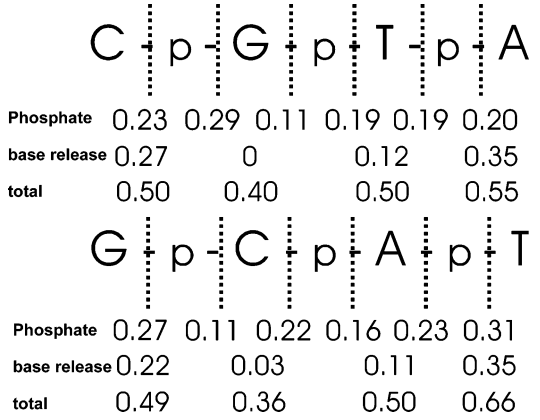
The presence of fragments containing a terminal phosphate was supported by treatment of the samples with alkaline phosphatase and comparison of the two chromatograms before and after treatment (Figures 1 and 2). Alkaline phosphatase removes the terminal 3′ or 5′ phosphate groups and, thus, converts fragments with a terminal phosphate to those without a terminal phosphate. In the case of CGTA, alkaline phosphate treatment led to the conversion of CGp to CG (peak 9 → peak 10), and so on, for the conversion of pTA to TA (peak 11 → peak 12), CGTp to CGT (peak 13 → peak 14), and pGTA to GTA (peak 15 → peak 16) (see connecting peaks between the middle and top chromatograms; Figure 1). The cleavage of mononucleotide fragments with a terminal phosphate was supported by the conversion of pA to dAdo (peak 7 → peak 8) and an increase in the yield of dCyd after treatment with alkaline phosphatase (peak 6). It should be noted that the peak area of fragments containing a terminal phosphate was smaller than the corresponding fragments without the phosphate. This effect may be attributed to peak tailing as a result of interactions between acidic silanol groups of the solid phase and charged terminal phosphate groups of nucleotide products. Similarly, treatment

of irradiated GCAT with alkaline phosphatase revealed the conversion of fragments with a terminal phosphate to those without the phosphate. For example, pAT converted to AT (peak 23 → peak 24), GCAp to GCA (peak 25 → peak 26), and pCAT to CAT (peak 27 → peak 28) (see connecting peaks between the middle and top chromatograms; Figure 2). Although GCp (peak 21) coeluted with an impurity under these conditions, the corresponding fragment without the terminal phosphate, GC (peak 22), greatly increased upon treatment with alkaline phosphatase. The separation of Gp (peak 17) and pT (peak 19) was poor under these conditions, and thus, the effect of alkaline phosphatase can be seen by an increase of the yield of dGuo (peak 18) and dThd (peak 20), respectively (Figure 2). The analysis of nonmodified fragments of CGTA and GCAT confirms the nature of phosphodiester bond cleavage induced by LEE which gives nonmodified fragments containing a terminal phosphate group.

Discussion

A major question in the mechanism of LEE-induced strand breaks in DNA is whether the electron initially localizes on the nucleobase, and then transfers to the phosphodiester bond, or whether direct electron localization occurs. According to density functional theory calculations and experimental evidence, at very low energies (<3 eV), there are two possibilities for the localization of LEE on DNA. One possibility involves direct attachment of the incident electron to a π^* orbital of the phosphate group forming a local transient negative ion (TNI).[15,23] The other possibility involves resonance capture of the electron by the lowest π^* orbital of one of the DNA bases, followed by electron transfer to the π^* orbital of the phosphate group.[24,25] The net result of both possibilities is the formation of a transition state with an extra electron in the usually unfilled P═O π^* orbital that can lead, via curve crossing to a σ^* anion state, to cleavage of the phosphodiester bond.[23,24] It is reasonable to assume that the same processes operate for electrons of 10 eV as well as for electrons of lower energy (0−3 eV). For example, Grandi et al.[26] have reported the formation of a shape resonance for uracil near 9 eV. This transient anionic state, which should also exist for DNA bases, could transfer its excess electron to unfilled orbitals of the phosphate group, lying at higher energy in a manner similar to that described for electrons of lower energy (<3 eV).[23] Furthermore, Martin et al.[15] compared their experimental results on single-strand breaks in plasmid DNA induced by 0−4 eV electrons with those on the damage imparted to isolated DNA bases by electrons of the same energies. The yield functions for strand breaks could be reproduced in magnitude and line shape by a model that simulates the electron capture cross section as it might appear in DNA owing to π^* anion states of the bases. The attachment energies and the magnitude of cross sections were taken from electron transmission measurements for gaseous bases.[27] The peak magnitudes were scaled to reflect the inverse energy dependence of the electron capture cross sections. Under the assumption that equal numbers of each base are resident in DNA, the contributions from each base were simply added. Although such a comparison suggests that electrons are initially captured by nucleobases, the formation of products will have to be studied by direct chemical analysis to test this hypothesis.[15]

Scheme 1. Distribution of Damage by Sites of Cleavage



The amount of cleavage at each phosphodiester bond may be estimated from the yield of each nonmodified fragment (Scheme 1). From these analyses, the yield of cleavage varied about 3-fold depending on the site of cleavage. If the electron was solely captured directly into the usually unfilled P═O π^* orbital, then according to the structure of oligonucleotide tetramers, there should be an equal probability of breaking the phosphodiester bond at all sites. As seen from Scheme 1, however, this is not the case; phosphodiester bond cleavage significantly varies from one site to the other. Therefore, we must conclude that electron transfer contributes to phosphodiester bond cleavage. Interestingly, the yield of phosphodiester cleavage was enhanced at the terminal positions of one of the tetramers (GCAT). The effect of the end was even more pronounced for base release although it is not known at this point whether base release arises from DEA-induced cleavage of the *N*-glycosidic bond or from DEA-induced cleavage of the phosphodiester bond (see below; discussion on base release). This end effect may be explained by the number of available sites for electron transfer such that electrons at internal nucleobases can transfer to two phosphodiester bonds, whereas those at external nucleobases can only transfer to one bond. In agreement with this hypothesis, the variation for the combined yield of cleavage at individual bases is reduced (see total; Scheme 1). Alternatively, the greater cleavage at terminal positions may be due to greater accessibility of terminal compared to internal positions toward initial electron attachment. For instance, LEE-induced desorption of CN and OCN fragments from a series of oligonucleotides was previously found to be inversely proportional to chain length (n < 9).[28] In summary, the present work shows that phosphodiester bond cleavage depends on nucleobase and sequence, but more studies will be necessary to clarify the nature of this phenomenon.

Once the electron has localized on the phosphodiester bond, there are two possible pathways leading to cleavage of this bond (Scheme 2). Pathway A involves scission of the C−O bond and gives carbon-centered radicals (C5′ or C3′ radicals) and

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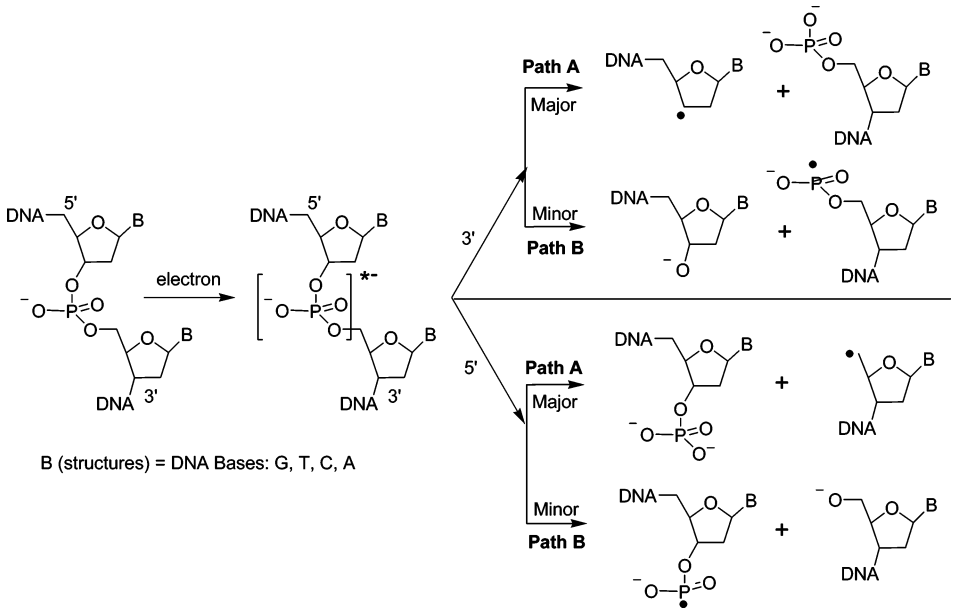
Scheme 2. Proposed Pathways for Phosphodiester Bond Cleavage of DNA



phosphate anions as termini, whereas pathway B results in cleavage of the P−O bond giving alkoxyl anions together with phosphoryl radicals. On the basis of our analysis of fragments from oligonucleotide tetramers, we demonstrate that cleavage of the phosphodiester bond primarily takes place via C−O bond cleavage leading to the formation of a sugar radical and a terminal phosphate anion (path A). The cleavage of C−O and P−O bonds, leading to the formation of phosphoryl radicals and dephosphorylated C3′ radicals of the sugar moiety, was previously reported in ESR studies of argon ion irradiated and γ-irradiated hydrated DNA.[29,30] Recently, the ESR spectra of both dephosphorylated C3′ and C5′ radicals were extracted from the neutral radical spectrum of irradiated DNA containing electron and hole scavengers.[31] In view of the greater bond dissociation energy of C−O (334 kJ/mol) compared to that of P−O (92 kJ/mol), it is remarkable that C−O bond cleavage was the dominating process on the basis of ESR studies (95%).[29] A possible interpretation of the latter result, in agreement with our results, is that the bond-breaking process takes place by electron attachment into an unfilled orbital lying at a much higher energy (i.e., 10 eV = 960 kJ/mol) than the thermodynamic threshold of C−O bond dissociation. In this case, phosphodiester bond cleavage would not depend on bond energy considerations but rather on the availability of dissociating anionic states at the energy of the captured electron.

The release of unaltered nucleobases from tetramers can be explained by two mechanisms of *N*-glycosidic bond cleavage. Previously, we showed that LEE (0−15 eV) efficiently induced the cleavage of thymidine to thymine.[17a] Similar results have also been reported for thymidine in the gas phase.[17b] Thus, it is reasonable to propose a similar pathway for the release of thymine (Thy) from tetramers and, as well, for the release of other nucleobases (Ade, Cyt, and Gua). On the other hand, base release may also occur by an indirect pathway involving the initial formation of sugar radicals resulting from DEA-induced cleavage of the sugar−phosphodiester C−O bond. It is well-known that sugar radicals lead to base release from the reaction of either OH radicals or sugar radical cations.[32,33] Assuming a similar conversion for sugar radicals from LEE-irradiated tetramers, the formation of nonmodified fragments should be comparable to the formation of base release products. Consequently, the sum of nonmodified fragments and base release products given in Scheme 1 may overestimate the total damage up to 2-fold. From our results, however, sugar radicals resulting from DEA-induced cleavage of the phosphodiester bond do not efficiently lead to base release at least at internal positions. For example, the release of Gua (∼0) and Thy (0.12) from internal positions of CGTA was much smaller than phosphodiester bond cleavage at G (0.29 + 0.11) and T (0.19 + 0.19) (Scheme 1). Similarly, the release of Cyt and Ade from GCAT was smaller than phosphodiester bond cleavage at C and A. Also noteworthy was the difference between base release preference at internal positions for LEE reactions (Thy ∼ Ade > Cyt > Gua; Scheme 1) and that for OH radical reactions (Cyt > Thy > Ade > Gua).[33] Thus, sugar radicals resulting from DEA-induced cleavage of the phosphodiester bond appear to have a different fate than sugar radicals produced by OH radical reactions; however, this may result from the fact that LEE-induced sugar radicals are generated in the condensed phase under UHV. In sharp contrast to LEE-induced base release at internal positions of tetramers, the yield of base release at terminal positions was severalfold higher despite a similarity in base release preference that existed when comparing the yields at terminal positions (Ade ∼ Thy > Cyt > Gua; Scheme 1). This difference indicates an important change in the mechanism of base release at the terminal position. One possibility is that sugar radicals resulting from DEA-induced cleavage of the phosphodiester bond lead to base release more efficiently when the radicals are formed at the terminal compared to internal positions. Another pos-

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sibility is that initial electron capture and/or transfer from DNA bases to the *N*-glycosidic bond leading to DEA-induced cleavage of this bond is affected by the absence of a neighboring base or phosphodiester moiety.

Similar to the irradiation of tetramers with LEE (this work), the irradiation of short oligonucleotides in crystalline form with 70 keV X-rays leads to the formation of strand breaks and base release as evidenced by analysis of oligonucleotide fragments.[34−36] The formation of these fragments in the latter studies was attributed to direct ionization and the formation of sugar radical cations. Interestingly, X-ray and LEE irradiation of oligonucleotides give similar results in terms of fragment product analyses. The major products in both irradiation systems included 3′ and 5′ end-phosphorylated strand fragments. Similar to LEE irradiation, there was also a preference for cleavage at termini positions in X-ray irradiation although this effect was not consistent in the study of several DNA sequences.[35] Whereas the distribution of phosphodiester cleavage was clearly affected by the nature of the nucleobase in LEE irradiation, there was little or no variation of cleavage with respect to nucleobases, as reported in studies with X-rays.[36] A more detailed comparison of irradiation products is warranted in future studies to determine the importance of LEE-induced cleavage reactions in the formation of damage to DNA by X-rays.

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Summary

The present work demonstrates that LEE induce phosphodiester bond cleavage and nucleobase release in oligonucleotide tetramers. LEE may directly localize on the phosphate group, but electron capture by other components followed by electron transfer to the sugar−phosphate backbone must also contribute to the rupture of phosphodiester bonds. The mechanism of phosphodiester bond cleavage is proposed to involve dissociative electron attachment (DEA). This mechanism entails two possible pathways of phosphodiester bond cleavage: one pathway involves the formation of carbon-centered sugar radicals with intact phosphate anions, whereas the other involves the formation of transient alkoxyl anions of the sugar moiety together with phosphoryl radicals. On the basis of product analysis, LEE irradiation of tetramers gave nonmodified fragments containing a terminal phosphate group, while those without a phosphate group were hardly detected. Thus, the mechanism of phosphodiester bond cleavage involves cleavage of the C−O bond rather than the P−O bond. In addition, we report the release of nonmodified nucleobases from irradiated tetramers; however, these products may arise from direct *N*-glycosidic bond cleavage or by an indirect pathway involving sugar radicals. The present study provides a chemical basis for the formation of strand breaks by the reaction of LEE with DNA.

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