DNA Damage Induced by Low-Energy Electrons: Electron Transfer and Diffraction

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Thin films of the short single strand of DNA, GCAT, in which guanine (G) or adenine (A) have been removed, were bombarded under vacuum by 4 to 15 eV electrons. The fragments corresponding to base release and strand breaks (SB) were analyzed by high performance liquid chromatography and their yields compared with those obtained from unmodified GCAT. From such a comparison, it is shown that, using GCAT as a model system, (1) most SB result from electron capture by DNA bases followed by electron transfer to the phosphate group and (2) the initial capture probability depends on the coherence of the electron wave within the tetramer.

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Introduction.—Since the discovery that low-energy electrons (LEE) inflict considerable damage to DNA [1], numerous attempts have been made to determine the precise mechanisms leading to fragmentation of this molecule [2]. It is now established that, for energies below 15 eV strand breaks (SB), and base release occurs essentially via the formation of transient anions located on DNA components (i.e., DNA base, sugar, and phosphate groups). The transient states can dissociate, or decay by electron emission, leaving the trapping molecular site unaltered or in an excited state. If the latter state is dissociative, bond scission can occur, so that both decay channels lead to damage DNA.

According to recent calculations [3], when the additional electron is released from a transient base anion, it can be transferred to the phosphate group, where dissociative electron attachment (DEA) leads to rupture of the sugar-phosphate C-O σ bond. Further quantum-mechanical calculations of electron scattering from and into DNA revealed that below 15 eV, electron capture by the bases can be strongly enhanced by constructive interference of the electron wave scattered within the molecule [4]. We show in this Letter, by direct analysis of the products induced by 4–15 eV electron impact on short single strands of DNA, that both mechanisms predicted theoretically are involved in the bond breaking process within the range 4–10 eV. Because LEE contain a large fraction of the energy deposited by high-energy particles [5], these results are relevant to a precise understanding of the mechanisms of the direct effect on DNA damage induced by high-energy radiation.

In recent experiments [6], we identified 12 fragments generated by 4–15 eV electron impact on the tetramer GCAT, shown on the left of Fig. 1. The fragments arise from cleavage of either the *N*-glycosidic bond next to one of the four DNA bases: guanine (G), cytosine (C), adenine (A), and thymine (T) (sites 1–4) or on either side of the repeated interconnecting phosphate-sugar backbone (sites 5–10). Rupture of the *N*-glycosidic bond at positions 1 to 4 causes base release, whereas scission of the phosphodiester C-O bond at 3′ (5, 7, and 9 in Fig. 1) and 5′ positions (6, 8, and 10) leads to a series of chain fragments. Quantification of the yields of fragments as a function of electron energy revealed that, below 15 eV, rupture of the *N*-glycosidic and phosphodiester bonds occurs essentially via the formation of transient anions located near 6 and 10 eV. Examples of yield functions from previous studies are shown in the lower portion of Fig. 2 for T, *p*T, and *p*CAT, where *p* represents a terminal phosphate group. These fragments correspond to breaks at positions 4, 9, and 5, respectively, in Fig. 1. All yield functions of the fragments from GCAT exhibit a broad peak around 10 eV, whereas the 6 eV resonance only appears in the yield functions of T, C, *p*T, G*p*, *p*AT, and *p*CAT. In the present work, we investigated the fragments produced by 4–15 eV electron bombardment of GCXT and XCAT where *X* represents a stable abasic site in which the base is replaced by a hydrogen atom. The nomenclature of GCXT is shown on the right in Fig. 1.

Experiments.—Five monolayer (ML) films of GCXT and XCAT were electron bombarded under ultrahigh vacuum (UHV) in a newly developed irradiator capable of producing sufficient amounts of products for *ex vacuo* analysis by high performance liquid chromatography (HPLC) [7]. Details of film preparation, electron irradia-

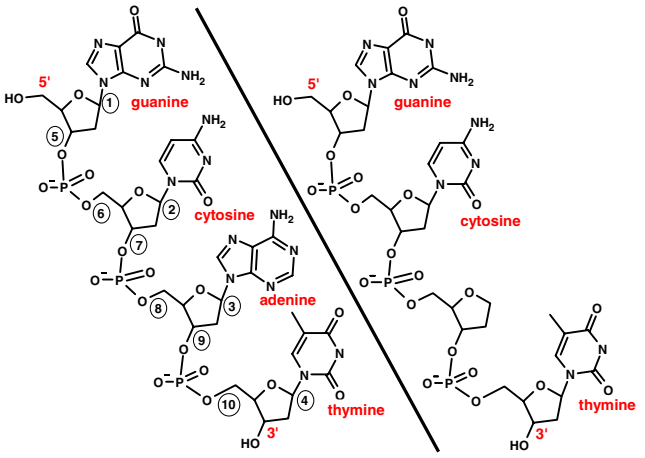

FIG. 1 (color online). The molecular structure of tetramers GCAT and GCXT (X = stable abasic site).

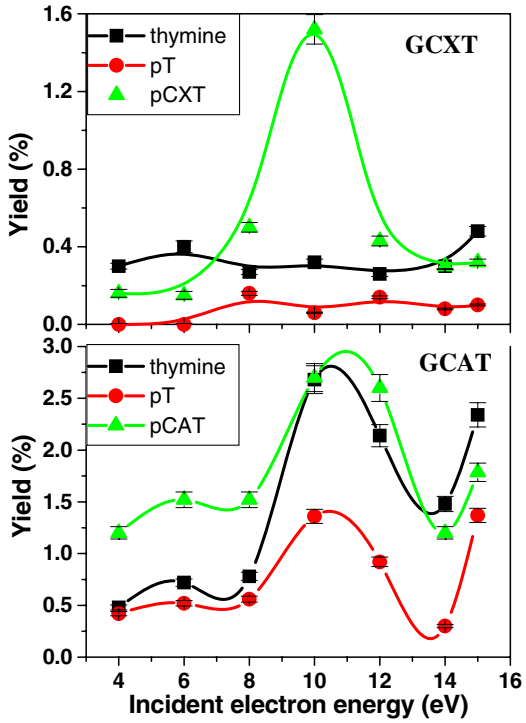

FIG. 2 (color online). Dependence of thymine, pT, pCXT, and pCAT yields on electron energy in GCXT vs GCAT.

tion procedure, and product analysis are given elsewhere [6]. The oligonucleotide tetramers and reference compounds were either purchased from Alpha DNA, Montreal, QC, or prepared by conversion of the tetramers into smaller fragments by reaction with P1 nuclease, DNAse I and/or alkaline phosphatase. All standard compounds were purified by HPLC with an ammonium acetate buffered solution, before further manipulation. The same solution was used for the preparation of GCAT, and thus, the counterion was NH_4^+ for all compounds referred to in this work. Eighty-five nmol of XCAT or GCXT were dissolved in 5 ml deionized water (Millipore) and the solution was deposited into seven chemically clean tantalum cylinders (9–12 nmol/cylinder). Films on the cylinder walls were produced by spin coating. The average thickness of the film was 2.3 ± 0.1 nm (5 ± 0.2 ML), as estimated from the average density of single strand DNA (1.7 g cm^{-3}) [8], assuming a uniform distribution of molecules.

The samples were transferred to the irradiation chamber, where they were individually bombarded by a uniform electron beam spreading over the entire inner surface of the cylinder. Under present conditions, the total electron density transmitted through the sample was 7×10^{14} electrons/cm^2. Five independent experiments were carried out for each energy of irradiation: in the case of GCXT at 4, 6, 8, 10, 12, 14, and 15 eV and for XGAT at 6, 10, and 15 eV. The accuracy of the energy scale was ± 0.3 eV. In each run, one of the samples was not bombarded and served as a control. From 4 to 15 eV, the irradiation time was adjusted to give an exposure well within the linear regime of the dose response curve and an equal number of electrons to each sample. All experiments were performed at room temperature.

After irradiation, the tantalum cylinders were removed from UHV and the tetramer and radiation products were recovered from the surface using a solution of 12 ml of degassed methanol and 1 ml H_2O. The sample was evaporated to dryness and redissolved in 200 μl of nanopure grade H_2O. Half of the sample was treated with alkaline phosphatase (AP) (Roche Applied Science) for 1 h at 37 °C to remove the terminal phosphate group of nucleotide fragments. The samples were then analyzed by HPLC. The mixture of products was separated on an ODS-AQ column (6 mm internal diameter $\times$ 150 mm length), 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 products were detected by UV absorption at 210 and 260 nm. The yield of LEE-induced products was determined by calibration with authentic reference compounds, as described previously [6].

In order to compare the yield of fragments from GCAT, GCXT, and XCAT, the relative solubility, deposition, and recovery was examined for each tetramer. From these experiments, we conclude that all of the tetramers are deposited and recovered with approximately the same efficiency. In addition, the exposure of single tetramers from independent experiments gave the same ratio of damage as that obtained by exposing an equal mixture of two tetramers.

Results and discussion.—With the exception of the missing base, the same fragments were observed in the mixture of products from irradiated GCAT tetramers with or without an abasic site. Table I provides a comparison of the yields expressed as the percentage of SB and base release from the initial amount of tetramer before bombardment at 6, 10, and 15 eV. Yield functions for GCXT were produced from such yields for all fragments recorded at seven different energies between 4 and 15 eV. Examples for the yield functions of T, pT, and pCXT clearly indicate that, with the exception of pCXT, the 10 eV resonance disappears (upper portion of Fig. 2). The signal at the 6-eV resonance in GCXT was too low to reach the same conclusion. More generally, the 10 eV resonance disappears in all yield functions for base release and appears only in yield functions corresponding to scission of the backbone at sites that are distant from the abasic site (i.e., in the yield functions pCXT, Gp, pXT, and GCXp, which corresponds to SB at positions 5, 6, 7, and 10, respectively, in Fig. 1). In other words, the 10 eV resonance at sites 8 and 9 associated with the abasic site disappears, whereas this resonance persists for damage at the other sites along the backbone. Thus, in GCAT, the formation of SB at position 8 and 9 via transient anion formation is due to the presence of adenine. This observation can only be explained by invoking electron capture by adenine in GCAT followed by electron transfer to the backbone of DNA.

TABLE I. Comparison of damage yield of tetramer at electron energies of 6, 10, and 15 eV. Original amount of tetramer = 16.8 nmol; standard deviation = 10%.

	6 eV			10 eV			15 eV		
Yield (%)	Strand break	Base release	Total	Strand break	Base release	Total	Strand break	Base release	Total
XCAT	0.72	0.39	1.11	1.11	0.45	1.56	9.89	1.14	11.03
GCXT	0.80	0.60	1.40	4.56	0.56	5.12	2.34	0.78	3.12
GCAT	4.76	1.96	6.72	9.54	5.92	15.46	10.30	4.72	15.02

Another mean to show that electron transfer occurs from DNA bases to the phosphate group is to express the yield of each fragment resulting from SB as a percentage of the total damage to a particular tetramer. Such a representation of the damage is shown in Fig. 3, where the percentage of fragments corresponding to bond cleavage at positions 5 to 10 is given for bombardment of XCAT, GCXT, and GCAT at 6, 10, and 15 eV. Quantitative comparisons of these numbers from one tetramer to another are difficult because the percentage of fragments is computed for each tetramer. However, it is obvious that at 6 eV, when G is absent (i.e., in XCAT), there is no cleavage of the phosphodiester bond at the position lacking the base moiety (i.e., position 5 in Fig. 1). Similarly, when A is removed (i.e., in GCXT), there is no dissociation of the C-O bonds on either side of A (i.e., at positions 8 and 9). Thus, at 6 eV, G and A must be present within GCAT to produce C-O bond rupture next to the base (positions 5, 8, and 9). It is difficult to explain this result without invoking electron capture by G and A followed by electron transfer to the corresponding phosphate group. This phenomenon is not observed at 10 and 15 eV, with the exception of bond rupture at position 9 which decreases from 10% in GCAT to 1% in GCXT at 10 eV; it corresponds to the disappearance of the 10 eV resonance in the yield of pT shown in Fig. 2, which may be attributed to inhibition of electron transfer from A to the corresponding phosphate group.

Since electron transfer from a DNA base π^* to a C-O σ^* orbital has been shown theoretically to occur at energies below 3 eV [3], we suggest that, in our case, the incident 6 eV electron electronically excites a base before transfer to the C-O orbital. Recent LEE energy-loss spectroscopy experiments on the DNA bases show that electronically excited states exist within the 3.5 to 6 eV range [9]. For example, LEE energy-loss spectra of thymine exhibit electronically excited states at 3.7, 4.0, and 4.9 eV ascribed to excitation of the triplet $1^3\,A'$ ($\pi \rightarrow \pi^*$), $1^3\,A''$ ($n \rightarrow \pi^*$), and ($\pi \rightarrow \pi^*$) transitions [9]. Excitation of these states by 6 eV electrons forming a core-excited shape resonance on T would produce electrons of energies below 3 eV, which could then transfer to the phosphate-sugar backbone. In other words, the resonance decays by leaving one hole and one electron in a previously empty orbital on the base and the excess electron via through-bond interaction is coupled to an empty σ^*CO orbital. We denote this decay channel as the ''electron transfer channel.'' Energy-loss electrons could also transfer into π^* orbitals of adjacent bases, which lie in the range of 0.29 to 4.5 eV [10], before transferring to the backbone. Thus, by resonance decay to the electron transfer channel following excitation of the bases, electrons having the energies in the range for transfer [3] would be created and lead to C-O bond scission. If the transient anion and/or the final electronically excited state on the DNA base is dissociative, it could lead to scission of the N-glycosidic bond, thus causing base release.

Returning to Table I, we now consider another striking aspect of these results: removing a base in GCAT causes a drastic reduction in the quantity of damage at 6 and 10 eV. For example, at 6 eV, SB are reduced by a factor of about 6 and base release by a factor of 3.3 and 5 for GCXT and XCAT, respectively. In a classical picture, where the damage caused by electron capture by DNA bases is simply additive, we would expect that the amount of SB and base release in XCAT and GCXT would decrease by ~25% (i.e., to 3.57% and 1.47%, respectively), if rupture of all the N-glycosidic and C-O phosphodiester bonds have the same

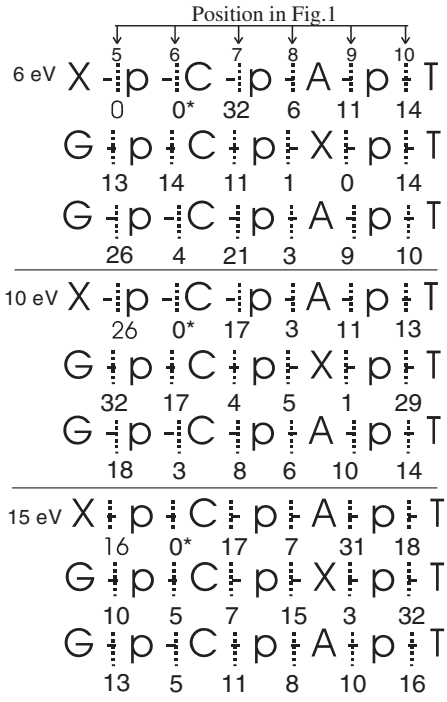

FIG. 3. Percentage distribution of strand breaks by sites of cleavage, induced by 6, 10, and 15 eV electrons. $^*Xp$ was not detected by HPLC and the yield was considered to lie below the detection limit.

probability. This nonlinear decrease in damage caused by introduction of an abasic site is also reflected in the yield functions from GCXT for all fragments recorded in the present experiments; in particular, the decrease in damage is huge upon disappearance of the 10-eV resonance, as seen in Fig. 2.

According to the results of Table I, the magnitude of damage in GCAT is caused by a collective effect involving DNA bases, which appears to be strongly suppressed by removal of G or A. In GCAT, creation of abasic sites modifies bond energies and the geometrical conformation of the molecule, due to changes in base stacking. In single-stranded oligomers, the effect of base π stacking on C-O sugar phosphate and *N*-glycosidic bond cleavage induced by attachment of electrons to C has been calculated by Anusiewicz *et al.* [11] for energies below 3 eV. The inclusion of π stacking causes an increase of the energy barrier to break these bonds. Thus, the creation of an abasic site that reduces stacking interactions should increase bond scission. This prediction, however, is contrary to observation. We must therefore consider that the initial electron-molecule interaction and hence the cross section for electron capture is highly sensitive to the number of bases and the overall topology of GCAT. Although we have no information on the topology of our tetramers on the surface of the tantalum cylinder, we know from recent calculations of LEE scattering from and within DNA that the ordering of DNA bases, in a helical configuration within the molecule, strongly influences the electron capture probability by these components [4]. More specifically, the magnitude of electron capture probability by DNA bases for partial waves of certain momenta has been found to increase up to 1 order of magnitude, owing to constructive interference of these partial waves within DNA. Since these interferences are related to the relative position of the bases and oligomer topology, they should be considerably modified when the stacking interaction is lowered by base removal. The differences in yields from GCXT (or XGAT) and GCAT could, in fact, result not only from the different nature of the base removed, but also from the different geometrical configuration of the two molecules. For example, according to this diffraction mechanism and the results in Table I, the structure of XCAT would not destroy constructive interference at 15 eV, thereby giving the expected decrease of about 25% less damage than in GCAT; but, this interference would be broken in GCXT, where the yield drops by a factor of about 5 compared to that from GCAT.

Introducing an abasic site in the tetramer could also considerably reduce interbase electron transfer, particularly in GCXT, where electron capture by T and C from a transient anion on A would be inhibited. However, even if we assume that all electrons captured by G are transferred to C, the yield of SB and base release would be reduced only by a factor of 2 in XCAT, which is insufficient to explain the data of Table I at 6 and 10 eV. Thus, we conclude that inhibition of interbase electron transfer could play an important role in the nonlinear decrease of damage due to base removal, but to explain the magnitude of this decrease electron diffraction must be invoked. Finally, we note that without electron transfer, SB could also arise from direct DEA to the phosphate group, but with a much reduced intensity, as shown in Fig. 2 and Table I. As previously observed, DEA in thin films of the phosphate group analog NaH_2PO_4 leads essentially to rupture of O-H bonds, within the 4–10 eV range [12]. The same bonds within the DNA backbone correspond to those linking oxygen with carbon atoms.

In conclusion, the yields of SB and base release induced by the impact of 4–10 eV electrons on the short DNA strand GCAT strongly depends on the initial electron capture probability of the DNA bases to form transient anions. This capture amplitude is highly sensitive to the number and possibly the geometrical arrangement of the bases. Most SB occur by electron transfer from a base to the phosphate group, causing rupture of the C-O bond via DEA. Owing to the universality of the law of quantum mechanics, we expect these basic mechanisms, responsible for LEE-induced damage in dry DNA, to be also operative in living cells. However, because of the different environment of the cell, the magnitude of this damage could be modified. Hence, studies such as those presented here remain to be validated under the hydrated and aerobic environment of the cellular medium to assess the importance of LEE-induced processes at the radiobiological level.

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