DNA strand breaks and crosslinks induced by transient anions in the range 2–20 eV

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Abstract

The energy dependence of the yields of single and double strand breaks (SSB and DSB) and crosslinks induced by electron impact on plasmid DNA films is measured in the 2–20 eV range. The yield functions exhibit two strong maxima, which are interpreted to result from the formation of core-excited resonances (i.e., transient anions) of the bases, and their decay into the autoionization channel, resulting in $\pi \rightarrow \pi^*$ electronic transitions of the bases followed by electron transfer to the C–O σ^* bond in the phosphate group. Occupancy of the σ^* orbital ruptures the C–O bond of the backbone via dissociative electron attachment, producing a SSB. From a comparison of our results with those of other works, including theoretical calculations and electron-energy-loss spectra of the bases, the 4.6 eV peak in the SSB yield function is attributed to the resonance decay into the lowest electronically excited states of the bases; in particular, those resulting from the transitions $1^3A'(\pi_2 \rightarrow \pi_3^*)$ and $1^3A''(n_2 \rightarrow \pi_3^*)$ of thymine and $1^3A'(\pi \rightarrow \pi^*)$ of cytosine. The strongest peak at 9.6 eV in the SSB yield function is also associated with electron captured by excited states of the bases, resulting mostly from a multitude of higher-energy $\pi \rightarrow \pi^*$ transitions. The DSB yield function exhibits strong maxima at 6.1 and 9.6 eV. The peak at 9.6 eV is probably related to the same resonance manifold as that leading to SSB, but the other at 6.1 eV may be more restricted to decay into the electronic state $1^3A'(\pi \rightarrow \pi^*)$ of cytosine via autoionization. The yield function of crosslinks is dominated by a broad peak extending over the 3.6–11.6 eV range with a sharper one at 17.6 eV. The different line shape of the latter function, compared to that of SSB and DSB, appears to be due to the formation of reactive radical sites in the initial supercoiled configuration of the plasmid, which react with the circular form (i.e., DNA with a SSB) to produce a crosslink.

I. INTRODUCTION

Since the discovery that electrons with energies below 15 eV could induce single and double strand breaks (SSB and DSB) in DNA, considerable efforts have been devoted to understanding the precise mechanisms responsible for the damage and its localization on specific bonds.^{1–5} Relevant experiments have included mass spectrometry^{1–6} and high

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resolution electron energy loss (HREEL) spectroscopy^{7–10} of the basic molecular components of DNA in their gaseous and condensed phases, as well as chemical analysis of the products resulting from low-energy electron (LEE) bombardment of multilayer and self-assembled monolayers (SAM) films of DNA.^{1–5} More recently, LEE bond cleavage in DNA origami samples have been visualized by Atomic Force Microscopy (AFM).¹¹ By comparing the results generated across these various experiments, it has been possible in many cases, to deduce the mechanism responsible for bond scission. It is now established that below 15 eV, electrons break DNA bonds essentially via the formation of transient negative ions (TNI) of the basic subunits (i.e., the bases, sugar, and phosphate groups).^{1–6}

Several theoretical advances during the last decade have also made it possible to arrive at a fairly good understanding of processes caused by adding a single electron to a ground-state site of a subunit or small segment of a DNA molecule.^{12–15} Owing to the simplicity of such an initial event and the availability of substantial computational resources, the interaction of sub-excitation ($E < 3$ eV) electrons could be fairly well described using methods of calculations of different complexity.^{12–15} However, above the energy of the electronic excitation threshold of the basic constituents of DNA ($E > 3$ eV), the exact mechanisms leading to bond rupture are more difficult to determine; this is largely due to the formation of core-excited resonances. Theoretical calculations of the formation of core-excited TNI and their decay into harmful products are presently quite limited owing to the complexity of performing calculations with multiple electronically excited orbitals. So far, calculations have only been performed for electron scattering or capture by a small DNA strand involving a single electron in a ground or excited molecular orbital.^{12–15} According to multiple scattering theory,^{16,17} coherent enhancement of the electron wave function initially scattered within DNA is relatively modest at 9–10 eV, but below 4 eV can reach one order of magnitude for $\ell = 2$ partial waves and two orders of magnitude for $\ell = 3$. In general, as the electron energy decreases, the electron wave function becomes larger and more delocalized and hence diffraction, which is structure dependent, becomes prominent.

Taking into consideration the amount of present experimental^{8–10,18–30} and theoretical evidence,^{12–17,31–35} Scheme 1 was proposed as the main routes leading to strand breaks in DNA.⁴ The incoming electron of energy E_0 is preferentially captured via a shape, core-excited or core-excited shape resonance of a nucleobase. At energies E_0 about 0.5 eV below that of the first electronic excitation threshold, only shape resonances can be formed on a base. Dissociative electron attachment (DEA) to a base is possible, but route 1 is also accessible for electron transfer to the phosphate group or simply autoionization into the elastic continuum. Via route 1, the CO bond of the DNA backbone is cleaved at the 3' and 5' positions: the incoming electron captured in one of the lowest π^* -orbitals of the bases³⁵ is transferred to an antibonding σ^* orbital of the C–O bond in the backbone of DNA, where it resides for a sufficiently long time to rupture that bond.

Above the energy of the electronic excitation threshold, core-excited types of resonances are expected to lead to DEA via route 2, causing base damage or release. Route 3 in Scheme 1 is the channel into which electronic excitation of a base can release a very low energy electron (e.g., $E = \sim -0.5$ to 1 eV with respect to the vacuum level taken as 0 eV). Usually, only core-excited and core-excited shape resonances can decay into this latter channel. As in route 1,

the electron can be re-emitted into the continuum or transfer within DNA to the C–O σ^* bond when $E > 0$ eV. For $E < 0$ eV, the electron can only transfer within DNA. The electron transfer probability has been calculated to be large at very low energies ($E < 1$ eV).^{31–34} Hence, since in such resonances the additional electron can be released with very low energies, this type of TNI is expected to favor the electron transfer mechanism explained in route 1, leading to SSB in DNA. However, in the case of route 3 the process can be accompanied or not by base damage. Interestingly, a similar mechanism of conical intersections of multidimensional potential-energy surfaces between excited and ground states has been proposed to account for the photostability of the Watson-Crick base pairs in DNA.³⁶ In summary, one should find the core-excited resonances of the DNA bases to be largely responsible for the initial electron capture above ~ 3 eV and the energy dependence of their capture cross sections to be reflected in the yield function of SSB.

Although our understanding of LEE-induced damage to DNA has progressed significantly in the past decade, the new information has never been assembled to interpret the behavior of the 2–20 eV yield functions of SSB and DSB in bacterial (i.e., plasmid) DNA. Furthermore, since the appearance of the first results,³⁷ other groups have produced SSB^{38–41} and DSB⁴⁰ yield functions from LEE-impact on different types of plasmid DNA. In the present work, we measure the yield functions for the formation of SSB and DSB induced by 1.6–19.6 eV electron impact on thin films of pGEM-3Zf(-), 3197 base pairs (bp) plasmid DNA, with the highest possible sensitivity and accuracy. Resonances are now clearly seen at 4.6 and 6.1 eV in the yield functions of SSB and DSB, respectively. The present yield function of SSB is compared with those recorded in other laboratories with different DNA configurations, so as to obtain a general overview of the data presently available, which are most pertinent to DNA damage. The resonance structure found in many different experiments is discussed and interpreted in terms of our present understanding of LEE-DNA interactions, so as to assess the electron-DNA resonance manifold causing DNA damage in the 3–20 eV region.

We also report the first measurement of the yield function for the production of DNA interduplex crosslinks (CL). During DNA replication, recombination, and mitosis, the potential exists for the induction of interduplex CL by ionizing radiation, since these processes involve close chain interactions between DNA duplexes.⁴² Thus, given the potential cytotoxicity of these lesions,^{42–44} and the large number of LEE produced by ionizing radiation,⁴⁵ it appears important to measure and interpret the yield function of LEE-induced interduplex CL.

II. EXPERIMENTS

A. Preparation of plasmid DNA

Plasmid DNA (pGEM-3Zf(-), 3197 bp) was extracted from *E. coli* DH5 and purified with a HiSpeed plasmid Maxi kit (QIAGEN). The purified plasmid DNA consisted of 97% supercoiled, 1% CL, and 2% concatemeric configurations. The concentration of DNA was determined by ultraviolet (UV) absorption at 260 nm with a BioTek Epoch spectrophotometer, according to 1 OD = 50 ng/ μ l. The quantity of impurities including protein and salts in the plasmid DNA were estimated by measuring the ratio of UV absorption at 260 nm to 280 nm. The ratio was 1.88, which corresponds to a purity greater

than 85%.⁴⁶ Samples having absorbed UV radiation were not used to prepare films. Fresh and identical DNA samples were taken for LEE bombardment.

B. Preparation of DNA films

The DNA samples were deposited on tantalum (Ta) substrates. They consist of 450 ± 50 nm films vacuum evaporated onto a 0.4 mm silicon wafer. The surface of Ta was cleaned in pure ethanol and ddH₂O and dried with a flow of dry nitrogen. 10 μ l of DNA solutions consisting of 320 ng of DNA were deposited onto the cleaned Ta surface. After freezing at -65 °C for 7 min in a glove box containing an atmosphere of pure dry nitrogen, the samples were lyophilized (freeze-dried) under a pressure of 7 mTorr by pumping with a hydrocarbon-free turbo molecular pump for 2 h. Assuming that the molecules were uniformly distributed on the surface of radius of 3 ± 0.5 mm and the density of DNA is 1.7 g cm^{-3} ,⁴⁷ the average thickness of the film was approximately 15 nm (5 monolayers (ML)). Such a thickness has been widely used in previously DNA-LEE experiments. Before being irradiated, the samples were transferred to the ultra-high-vacuum (UHV) chamber of a LEE irradiator and kept there for ~24 h, until a pressure of about 2×10^{-8} Torr was reached at ambient temperature. This procedure extracts most of the water from DNA, leaving structural H₂O (i.e., 2.5 water molecules per nucleotide remain bound to DNA).^{48,49} The loss of water of hydration compresses the DNA into the A-form. A-DNA is one of three biologically active double helical structures along with B-DNA and Z-DNA. It is a right-handed double helix similar to the more common and well-known B-DNA form in solution, but with a shorter and more compact helical structure, and a larger number of base pairs per rotation.⁵⁰

C. LEE irradiation

The LEE irradiator, recently built at Fuzhou University, is similar to the one constructed and described by Sanche.⁴ Beam characteristics were modified to obtain a more uniform current density on the sample, particularly at very low energies where, owing to space charge, the current density may decrease. First, the LEE gun produces a wider beam at normal incidence on the film surface with a constant radius of 15 mm, over the 2–20 eV range and an energy resolution of 0.3 eV FWHM. The beam always covers an area larger than that of the DNA sample of approximately 12 mm². Second, the irradiator incorporates a double electron detector consisting of two concentric stainless-steel plates with respective surface areas s and S of 12 and 108 mm², which can be located at the position of the sample. By positioning the beam so as to maximize the ratio I_s/I_S of the current on plates of area s and S , one obtains the highest and most uniform current density on the sample. Assuming a Gaussian distribution for the beam, variation of the electron current on the DNA film is estimated to be less than 10%.

By changing the potential between sample target (ground) and the center of the filament of the LEE gun, the electron energy can be varied from 2 to 20 ± 0.3 eV. At each energy, the films were bombarded with an incident electron beam of 6 nA and exposures of 5 to 60 s. Four control samples were placed into the irradiator chamber and left unirradiated, to serve as the data point at zero fluence in the corresponding exposure-response curves. The energy scale was calibrated within ± 0.5 eV by taking, as the zero electron energy reference, the onset of electron transmission through the films. At this onset, the potential measured

between the point of emission of the filament and the substrate was +0.4 V, indicating the potential change due to the differences in work function between the connections in our apparatus and the DNA-vacuum interface. Hence, the absolute electron energy was obtained by subtracting 0.4 V from each voltage measured between the filament and the metal substrate.

Based on the average film thickness of 15 nm, which is of the order of the penetration depth (5–20 nm) of 4–15 eV electrons in liquid water or amorphous ice,⁵¹ many of the electrons impinging on the film should be transmitted to the metal substrate. However, due to local variations in thickness, some electrons are expected to be retained a sufficiently long time in the film to charge it. As recently shown, film charging does not affect the yields obtained from the slope at zero fluence of exposure-response curves,⁵² but can affect calibration of the energy scale. Furthermore, variation of potential along the film surface degrades the energy resolution of the beam.

D. Quantification of structural forms of DNA in the films

After irradiation, the DNA films were recovered from the Ta substrates by dissolving with 10 μ l of ddH₂O. Quantification of the different structural forms of DNA in the samples was performed by agarose gel electrophoresis as previously described.⁵³ The forms analyzed were the supercoiled (SC), nicked circular (C), linear (L), and crosslink (CL) configurations, which correspond to the initial configuration of the plasmid, SSB, DSB, and crosslinking between two DNA molecules, respectively. The DNA samples and the agarose gels were stained with SYBR Green I in the concentration of 100X and 10000X, respectively. The samples were run on 1% and 1.3% agarose gels in 1X TAE buffer at 100 V for 7 min following by 75 V for 68 min (5 V cm⁻¹). The gels were then scanned by a STORM 860 scanner (Molecular Dynamics) adjusted for the blue fluorescent mode at an excitation wavelength of 450 nm and PMT voltage of 800 in the normal sensitivity mode. The configurations of DNA were analyzed by the ImageQuant 5.0 (Molecular Dynamics) software. However, instead of using the automated peak-analysis program, each band in a given scan of the gel was deconvoluted manually and separated from the residual baseline, to obtain the most accurate percentage value possible for each configuration. The binding efficiencies of SYBR Green I for the same amount (80 ng) of SC and L DNA were measured and then the correction factor was determined to be 1.4. This factor arises from the weaker binding of SYBR Green I to SC DNA than to the C and L forms.

The SC, C, and L forms were identified according to their position of migration in the gel as determined from previous analysis.⁵³ The CL resulting from irradiating pGEM-3Zf(-) DNA were identified by Cai *et al.*,⁴² by reaction of the products with EcoRI and PvuI restriction enzymes. At low fluence crosslinking occurs essentially between the most abundant DNA forms present in the film; i.e., the SC and C configurations. On the electrophoresis gels, the SC-SC CL migrate to the same position as the concatemeric form and hence its magnitude could not be unambiguously determined. Another band, which appeared on top of the gel, was assigned to the only other plausible configuration (i.e., the SC-C CL). The magnitude of the SC-C band is reported in the present study.

III. RESULTS

Representative exposure-response curves for the formation of CL, SSB, DSB, and the loss of the initial SC configurations, induced by 9.6 eV electron impact on 5 ML DNA films are shown in Figure 1. Such curves were recorded at 11 different electron energies within the 1.6–19.6 eV range. Each data point in yield vs fluence curves is the average of four independent experiments. Thus, 52 electron bombardments and 8 control samples were required to produce the data of Figure 1 for a total of 720 samples over the entire energy range. This is required to extrapolate the exposure response to zero fluence, since the yields are not linear with exposure owing to a number of factors, including depletion of the initial targets and film charging. Extrapolation to zero fluence coupled with low incident current density is expected to produce initial slopes representing the most accurate values of the yields per incident electron possible. Furthermore, this procedure automatically eliminates any background contribution arising from manipulation of the sample. Effective yields per electron per DNA molecule measured in this manner are given in Table I for different electron energies. The standard errors are calculated from a linear regression fit analysis of the slope near zero fluence. The behavior of the exposure-response curve for the formation of CL is similar to that of the circular form; such a behavior is expected in the formation of SC-C CL, since their yield depends on that of SSB. On the other hand, the yields of DSB vs fluence better fit a linear function than an exponential one. This behavior is due to the fact that the initial targets available to produce the L form are not being depleted at the same rate as the fluence increases, since both the C and SC forms are sources for DSB. Thus, as shown previously by mathematical analysis,⁵² the linear behavior of the DSB exposure-response curve indicates that the production of L DNA is not related to two consecutive SSB, but is the result of a single electron interaction. The exposure response curve for the loss of the SC configuration, which combines the sum of all damages, reflects essentially the inverse of the SSB curve. This is expected since a SSB is, by far, the most prominent type of structural damage.

Strong variations are observed with electron energy in the yields of all configurations (Table I). With the exception of DSB, the highest yields are observed at 9.6 eV. These variations appear more clearly in Figs. 2 and 3, where the yields in Table I are plotted as a function of electron energy between 1.6 and 19.6 eV. Two strong peaks are located at 4.6 and 9.6 eV in the SSB and loss of supercoiled (LS) yield functions in Fig. 3. The dotted line in the graph of SSB and LS indicates that these two peaks are superimposed on a slowly, but continuously, rising background, which is fairly constant below 14 eV (Fig. 3). Such a background could arise from the overlap of the 4.6 and 9.6 eV peaks, but we cannot rule out that a non-negligible portion of the yields of SSB and SC loss may be featureless over the entire energy range. Below 4 eV, however, other resonance features have been recorded by Martin *et al.*²⁰ with maxima at 0.8 and 2.2 eV. To resolve these peaks more data points per eV are needed, as well as lower energies than possible in the present experiments. Thus, the 0.8 and 2.2 eV resonance peaks probably constitute the main contribution to SSB below 4 eV in the curves of Fig. 3. A yield function similar to that of SSB is obtained for the formation of DSB (Fig. 2), but the low energy peak has the largest magnitude and is shifted to 6.1 eV. The positions of maxima in the yield function of CL in Fig. 2 cannot be

determined, owing to the large statistical variations in these measurements. Within experimental errors, a plateau in those yields is observed within the 3.6–11.6 eV region with a peak around 17.6 eV.

In Fig. 4, we compare the results obtained by different laboratories on different types of damages induced by LEE impact on dry DNA films in vacuum. Conditions that may differ from one experiment to another include the purity and nature of the DNA, substrate, preparation, uniformity and thickness of film, electron-beam current density and fluence, and method of analysis. These experimental conditions are listed in Table II, along with other pertinent information. It is readily seen from Fig. 4 that such varied conditions still give rise to a striking resemblance in yield functions from A to E.

The results in A were recorded by the group of Fromm with plasmid DNA (pUC21, 3151bp) uniformly deposited on highly oriented pyrolytic graphite by intercalating between each molecule doubly charged 1–3 diaminopropane (Dap^{2+}).³⁸ The affinity of Dap^{2+} for DNA allowed the layer by layer growth of plasmid films of uniform thickness that could be measured and monitored by AFM. Owing to the protein-like NH_3^+ group of Dap^{2+} , the binding to DNA in these films is similar to that with proteins in the chromatin of the cell nuclei. The result shown here in A is the yield function for the loss of the SC configuration.

The results in B and C were recorded at the University of Sherbrooke. Curves in B exhibits the yield functions corresponding to release of thymine (T) and strand breaks producing pT and pCAT, from a short DNA strand, i.e., the tetramer GCAT,⁵⁴ where G represents guanine, C cytosine, A adenine, and p the sugar-phosphate group. The films were produced by spin coating under vacuum and transferred to UHV for LEE bombardment. The products were analyzed by high performance liquid chromatography (HPLC). Curve C represents the yield of H^- anions desorbed by electron impact on a film composed of synthetic 25 bp doubly stranded DNA and the pGEM 3199-bp plasmid.⁵⁵

The remaining curves D to G represent the yield functions for the production of SSB obtained in four different laboratories. Curve D is the initial data published by Boudaiffa *et al.*³⁷ and Huels *et al.*⁵³ Curve E is the present result recorded at the University of Fuzhou. Curve F was recorded with p14 6360-bp plasmid DNA by the group of Orlando under dry conditions.³⁹ This group has also reported the LEE yield function for SSB with DNA under hydrated conditions.⁴⁰ In that case, DNA adopts the B form and the energy dependence is different than that of curve F; the structures have a tendency to shift to higher energy in agreement with the results of Ptasi ska and Sanche⁵⁶ The bottom curve G was produced in the group of Kumar by LEE impact on 5-ML thin films of plasmid PQE30 DNA.⁴¹

As seen from Table II, the energy scale was not always calibrated with respect to the vacuum level; in many instances, the electron energy was simply taken as the potential energy difference between the electron source and the substrate. Also, most of the data was recorded at intervals of 1 eV or larger. The energies of the maxima in Fig. 4 could therefore easily differ by ± 1 eV. With the exception of the experiment that produced curve F, the films were sufficiently thin and the incident current density and fluence sufficiently low to avoid appreciable charging of the film. However, owing to irregular film thickness,⁵⁷ the number

of electrons trapped at different position in the film can vary considerably depending on preparation. This variation of potential reduces the electron beam resolution and could smear resonance features and produce different line shapes. Furthermore, in order to insure that the yields are the result of a single electron interaction the fluence must be kept in the linear portion of the exposure-response curve. With the exception of the experiments, which produced curves D and E, exposure-response curves were not recorded. Judging from the present results of Fig. 1, which are linear up to about 3×10^{11} electrons (i.e., ~ 4 electrons/DNA), we can conjecture that most curves in Fig. 4 may contain damage induced by two or more electrons. One way to produce only single-hit damage is to bombard the film, not only with very low current density, as in the case of curve G, but also with low fluence. The disadvantage is usually the appearance of large statistical variations in the yields. Another method is to record the fluence-response curve and extrapolate the yields to zero dose as it was done to produce curves D and E. In this case, better statistics are obtained, but data acquisition becomes time consuming.

Nevertheless, despite the large variations in the experimental conditions that produced the data of Fig. 4 and the different types of DNA and measurements, curves A to F clearly show the predominance of a strong maximum lying between 9 and 11 eV. Another peak is also perceptible between 4.5 and 6 eV in the curves of A, B, and E. A shoulder is also present in this energy region in the data of Boudaiffa *et al.*³⁷ (curve D). In the light of the present results, this shoulder appears to be an unresolved peak, possibly due to a reduction in the resolution of the electron beam caused by fluctuation of potential along the surface of the film (i.e., non-uniform distribution of electrons locally trapped at the surface, owing to presumably larger fluctuations in film thickness in the experiment of curve D than in that of curve E). In fact, electron trapping is expected to be more intense in the case of curve D, since it was recorded with higher incident currents. A similar explanation can be given to account for the broader 10-eV structure in curve D compared to that of curve E. Another region can also be delineated from curves D and G where a peak appears between 14 and 16 eV. According to the data of Fig. 2, a structure is also present in the yield functions of CL at slightly higher energy. Finally, we note that in Fig. 3 both yield functions for SSB and LS are similar, since the main products of LEE-induced damage are SSB. Thus, the difference between curves D and E appearing in Fig. 4 are also found in the comparison of the LS yield function recorded in our experiment with that previously recorded by Boudaiffa *et al.*³⁷

IV. DISCUSSION

As seen from Fig. 3, the yields of SSB are still substantial below 4 eV down to 1.6 eV. They are considered to arise from pathway 1 of Scheme 1 described in the Introduction. This pathway involves the 0.8 and 2.2 eV shape resonances previously observed by Martin *et al.*²⁰ in the yield function of SSB recorded with the same plasmid as in the present experiments. This “background” yield appears to be progressively replaced by the signal from overlapping of the 4.6 and 9.6 eV resonance peaks, although the possibility of direct electronic excitation leading to bond dissociation cannot be completely rejected.

The resonance features in the 4–6 eV and 9–11 eV regions of the yield functions of Fig. 4 can be interpreted as due to the initial formation of core-excited or core-excited shape

resonances. The reasons for this assignment arise from basic considerations and general gas-phase data. Contrary to shape resonance, core-excited resonances have long lifetimes above 4 eV and can more easily couple to electronically excited states.^{58–60} The latter coupling is particularly effective in the case of a core-excited shape resonance, that usually lies 0–1 eV above the energy of its parent electronically excited state, into which it strongly decays. In such a resonance, an electron is captured by both a centripetal potential and the positive electron affinity of an electronically excited states.

At the experimental level, Allan and co-workers^{61–65} and others^{66–72} observed DEA from core-excited TNI states in the anion yield functions of some 35 hydrocarbons, including large alcohols and esters as well as saturated compounds containing ether and hydroxyl groups. Using the energies of the potential grand-parent cation state (i.e., the positive ion core) as a guide, Allan *et al.*^{63–65} could assign the peaks in their 5–12 eV anion yield functions to the initial formation of core-excited resonances with a hole in either the oxygen nonbonding orbitals or in one of the many σ orbitals. Comparison with photoelectron spectra allowed the nature of the resonance process and the binding energy of the two electrons in Rydberg orbitals around the positive ion core^{64,65} to be established. The σ -type core-excited resonances lying in the 8–11 eV region of large hydrocarbons, which decayed via DEA, did not depend on the nature of the molecule.⁶¹ Thus, their result corroborated the hypothesis that LEE interacting with even larger molecules such as DNA might also localize around specific bonds or subunits to form core-excited resonances.⁶¹

As explained in the Introduction, an electron impinging on DNA with energies commensurate with those of core-excited resonances is likely to be captured by a base^{4,35} and afterwards transfer to the phosphate group, via route 3 in Scheme 1, where it occupies the dissociative σ^* orbital of the C–O bond. We therefore expect the yield function for SSB to follow the line shape of the energy dependence of the electron capture cross section of the bases and hence find the resonance peaks in the SSB yields of Figs. 2–4 at the energies of the core-excited resonances of the bases. These energies have been measured for gaseous^{73–80} and condensed^{8–10,81,82} nucleobases. The latter energies, being affected by the polarization of the medium and other processes that modulate the energies of TNI in the condensed phase, it is more appropriate to compare data from DNA films with these; so we present in Table III a list of the most intense core-excited resonances found in different decay channels for the four DNA bases in condensed films. The resonance energies are given in the second column. The channels in which they were observed in HREEL and ESD experiments are reported in the right column. In the case of decay by autoionization, leaving the base in an electronically excited state, the resulting transition is given, whereas for the DEA channel, we refer to the maxima in each specific anion yield function. Staking of the bases within DNA probably slightly modifies the energies of the resonances in Table III. However, the attractive π - π non-covalent interaction between the bases is present in the tetramer GCAT, for which DEA peaks lie within the range 9–11 eV,^{82,83} as shown in the bottom lines of Table III. We find essentially three groups of core-excited resonances (I, II, III) located in the 5–6 eV, 8.5–11 eV, and 13–16 eV regions, respectively. These ranges agree well with the energy windows shown in Fig. 4 for the resonances observed by many groups in various yield functions. According to Table III, three-quarters of the resonance-

decay channels of the bases lies in the 8.5–11 eV region. It is therefore not surprising to find that the strongest yields in SSB are located in this energy range.

In Fig. 4, the core-excited resonances lying within the 4–6 eV region correspond to those located at 5 and 6 eV in Table III (i.e., group I), which decay by autoionization leaving the bases thymine or cytosine in their lowest triplet electronically excited states. According to Scheme 1, after creating these states, the autoionizing electron returns to the continuum (e_c) or transfers to the phosphate group (e_p) causing a SSB. The strong resonance structure seen in yield functions A to F between 8.5 and 11 eV in Fig. 4, probably arises from contributions of the many core-excited resonances listed as group II in Table III. Most of the autoionization decay channels (i.e., route 3 in Scheme 1) lead to higher lying $\pi \rightarrow \pi^*$ transitions. From their width in the HREEL spectra, many of these TNI states could be dissociative in the Franck-Condon region and, depending on their lifetimes, give rise to ESD of anions. In other words, within DNA, routes 2 and 3 in Scheme 1 may be available simultaneously. Table III also lists the energy of the resonance peaks found between 8.5 and 11 eV (Group II) in ESD yield functions of the anions H^- , O^- , CN^- , OH^- , OCN^- observed to desorb from thin films of the bases⁸¹ and the tetramer GCAT.^{82,83} In GCAT, the H^- signal is by far the strongest; it can be compared to that producing curve C with a peak near 10 eV in Fig. 4, which has been interpreted as arising from DEA to the bases (route 2 in Scheme 1) with a possible smaller contribution from the sugar ring.⁵⁵ Recent experiments on thymine and uracil deuterated and methylated at the various positions indicated that even at 8.5 eV, H^- loss is not only bond selective but also site selective.^{74,79,85} According to these experiments, the higher energy features around 9.3 eV in condensed thymine and GCAT could be caused primarily by the H^- loss from the C atoms with a contribution of H^- loss from CH_3 .^{82, 83} Similar findings were also reported in other studies,⁶⁹ for DEA to small organic molecules, i.e., acetic acid, propanoic acid, and *n*-propyl amine, which contain the methyl group. Thus, the pronounced peaks near 10 eV in C of Fig. 4 and at 9.2 eV in the H^- yield function from GCAT possibly arise essentially from H^- production at the carbon sites of the nucleobases. As previously explained, anion ESD is often inhibited at low energies in the condensed phase, due to the attraction created by the polarization potential induced by the TNI. In gas-phase studies, the 4–6 eV DEA resonances producing H^- below 6 eV are due to N–H bond cleavage at the N1 and N3 position of thymine and adenine.^{84–86} The largest DEA signals arise from thymine at 6.8 eV and a strong contribution is also seen in the DEA yields of CN^- giving a peak at 6 eV. The formation of OCN^- and CN^- anions from nucleobases requires a complex pathway possibly involving multibond cleavage driven by the high electron affinity of OCN (3.61 eV) and CN (3.82 eV).^{70,76,82} The formation of the anions mentioned in this paragraph and their corresponding neutral radicals, do not directly contribute to strand breaks, but the reactive sites created by DEA to the bases could directly or indirectly be involved in the production of CL.

Another resonance region between 14 and 16 eV can be identified from Fig. 4. The resonance feature, which is the strongest feature in the yield function G, was also seen at the same energy in the original yield function of SSB recorded by Huels *et al.*⁵³ and Boudaiffa *et al.*³⁷ Although its existence is less certain from the data shown in Fig. 4, we note that the yield function for CL also exhibits a maximum at a slightly higher energy. As seen from Table III, another group (III) of core-excited resonances exists in the DNA bases between

about 13 and 16 eV, which correlates well with the 14–15 eV structure in yield functions D and G of Fig. 4. These resonances have only been observed in the DEA decay channel. Depending on the branching ratio between pathways 2 and 3 in Scheme 1, the DEA resonances seen in ESD from films of the condensed bases could be involved in electron transfer to the phosphate group and induce a SSB.

Although a theoretical description of the core-excited resonance manifold of the nucleobases is not available, the work of Winstead and McKoy⁸⁷ provides evidence for the formation of core-excited resonances of the bases in the 4–11 eV region. These authors used Schwinger multichannel methods to carry out static exchange plus polarization calculations of electron scattering from pyrazine; a benzene-like ring molecule that contains a nitrogen atom and is simpler, yet similar to the nucleobases.⁸⁷ The two lowest-energy resonances were nearly pure single-channel shape resonances, but the highest-energy one was heavily mixed with a core-excited configuration built on the low-lying triplet states. The partial cross section for electron-pyrazine scattering in the resonant $^2B_{2g}$ symmetry exhibited strong maxima between 4 and 6 eV region, depending on different treatments of polarization. The lowest-energy excited state of pyrazine arises from the $\pi_2 \rightarrow \pi_1^*$ and $\pi_3 \rightarrow \pi_2^*$ transitions, giving a $^3B_{1u}$ symmetry. If this state temporarily attaches an electron in the lowest empty orbital, the resulting core-excited resonance takes the B_{2g} symmetry. The third excited state $\pi_3 \rightarrow \pi_2^*$ $^3B_{2u}$ can also attach an electron in π_2^* to form a B_{2g} core-excited resonance. According to these calculations, one would expect with efficient electron transfer to the phosphate group, to find within the 4–6 eV range a resonance peak in the DNA SSB yield function associated with transitions to π_3^* orbitals of thymine. As seen from Table III, the decay channels of the lowest core-excited resonance in thymine have been identified to correlate with the $\pi_2 \rightarrow \pi_3^*$ and $n_2 \rightarrow \pi_3^*$ transitions.⁸ Later, Winstead *et al.* calculated the cross sections for elastic electron scattering by cytosine, thymine, 2'-deoxycytidine, and 2'-deoxythymidine.⁸⁸ They found that disagreements between their results and experiment was largest for the third shape resonance involving a $\pi \rightarrow \pi^*$ transition around 10 eV. The discrepancy was attributed to the mixing of the third anion state with core-excited resonances built on one or more of the low-lying triplet states.⁸⁸

The strong peak around 10 eV in the curves of Fig. 4 is also present in the yield function of DSB shown in Fig. 2. It corroborates the results obtained by Huels *et al.*⁵³ and Boudaiffa *et al.*³⁷ with the same plasmid and appears to be associated with the group of TNI labeled as II in Table III. However, the other peak near 6.0 eV was not seen in their yield function. The shift of the low-energy resonance from 4.6 to 6 eV in going from SSB to DSB seen in Figs. 2 and 3 may be indicative of the predominant role of the core-excited resonance at 6 eV in cytosine, which decays by autoionization, into the $^3A'$ ($\pi \rightarrow \pi^*$) excited state channel. Thus, cytosine may be preferentially involved in the mechanism causing the rupture of two adjacent DNA strands. However, since our knowledge of the mechanisms leading to a DSB with a single electron hit is presently quite limited, it would be highly speculative to make any specific assignment on any mechanism that can lead to such double bond scission. As explained in the Introduction, the efficiency of intrastrand electron transfer to cause a SSB in DNA is well established both theoretically^{31–34} and experimentally.^{21–24,29} If *interstrand* electron transfer occurred with a similar efficiency, it would easily cause DSB with single-electron interactions. In the simplest case, a core excited TNI would have to form directly on

the phosphate group and decay by autoionization, leaving the CO unit in the backbone in an electronic excited state that would dissociate breaking the CO bond. Simultaneously, the departing electron would transfer to the opposite strand and form a transient anion of the adjacent phosphate group causing CO bond rupture on the other chain by DEA. However, such a mechanism would not involve initial electron capture by a base and the core-excited resonances shown in Table III, unless the unlikely transfer of two excited electrons and a hole occurs. Direct electron capture by the phosphate group at the terminal nucleoside has been demonstrated in short oligonucleotides,²² but is expected to occur with low probability in a long DNA strand. Obviously, more theoretical input is needed to determine the mechanism of DSB formation by a single LEE.

The overall line shape of the yield function of CL in Fig. 2 suggests that the 4–12 eV region is governed by non-resolved overlapping resonances and/or direct electron scattering. Crosslinking between SC and circular DNA requires these configurations to be in close proximity. A radical created on either forms then interacts with the other form creating a CL. If the reaction occurred only from an open bond in the circular configuration, the SSB and CL yield functions should have a common initial mechanism of formation, and therefore similar line shapes, unless the radicals formed at the different energies had very different crosslinking abilities. According to the comparison between of Figs. 2 and 3, the line shapes of the SSB and CL yield functions are very different. It is therefore possible that the CL yield function reflects mostly the energy dependence of radical formation on SC DNA and their reaction rates. Such radical formation is not limited to electron capture by the bases; so *a priori*, the CL yield function is not expected to be the same as that of SSB.

Finally, we note that electron capture by a base is strongly dependent on the probability of the incoming electron to be scattered along the axis of the DNA molecule.⁸⁹ Hence, all of the results presented in this article could be influenced by this phenomenon. Multiple LEE scattering within A-form DNA has been calculated for electrons incoming at normal incidence to the axis, an orientation, which is expected to correspond to the conditions of the experiments described in Sec. III; i.e., conditions where the molecule should lie more or less parallel to the surface plane of the substrate. Owing to the regular ordering of the bases, diffraction occurs within the coherence length of electron waves propagating in the axial direction.^{89,90} As a result, the probability of scattering along the axis is highly dependent on the energy of the incoming electron and exhibits strong maxima around 5–7 eV and 9–11 eV in the normal incidence elastic scattering function. Thus, interference of electron waves within DNA may also contribute to the line shape of the yield functions of Figs. 2–4.

V. CONCLUSION

In the past decade, numerous experiments and calculations have been performed on LEE-interaction with the DNA bases, the sugar analogs, the phosphate group, and longer moieties of DNA in an effort to explain how LEE, produced in copious amounts by ionizing radiation, damage the genome. The main goal of the present work has been to address this initial problem with the information now available, while providing new measurements of LEE-induced CL and SSB for comparison with previous results. Discrepancies have been found between various measurements, which may be related to the purity and nature of

DNA, substrate, preparation, uniformity and thickness of film, electron-beam current density and fluence, and method of analysis. Even when recorded with the same plasmids obvious differences still appeared in the yield functions of SSB. These were attributed to local variations of film thickness, which unevenly charged the film and thus reduced the electron beam resolution. Despite these differences, nine out of ten yield functions for different types of DNA damages recorded over the last 14 years,^{37–39,41,54,55} exhibit a strong maximum around 10 eV. A weaker structure also appears in four of these functions close to 5 eV, whereas in the earlier results of Boudaiffa *et al.*³⁷ a shoulder is present around this energy. The 5 and 10 eV features could be correlated to known core-excited resonances of condensed DNA bases in the 5–16 eV range. As previously suggested,⁴ after electronic excitation of a base, the energy-loss electron can transfer to the phosphate group, where it occupies temporarily a previously unfilled dissociative σ^* orbital of the C–O bond, causing a SSB. A decisive conclusion on the mechanisms involved is still awaiting theoretical calculations of core-excited resonance formation on the basic subunits of DNA and short DNA strands. The yield function of DSB appears to be related to that of SSB, but the mechanism leading to two strand breaks with a single electron, within a distance of about 10 bp is more complex. To determine the exact mechanism leading to DSB more refined experiments, where the electron transfer rates to specific subunits within DNA could be detected and possibly modified, must be elaborated. In contrast, the yield function of CL does not appear to be related to that of SSB, indicating that the initial electron capture mechanism is different from that breaking a single strand. The SC-C crosslinking monitored in the present experiment, probably arises mainly from more general damage to SC DNA, followed by reaction the damage SC with the circular configuration induced by LEE impact.

Understanding the action of LEE on DNA is of particular value in the development of new radiosensitisers and chemoradiation therapy protocols.^{91–96} As shown in the case of platinum chemotherapeutic agents, these drugs can become radiosensitizers, with appropriate timing between intravenous injection and irradiation, by preferentially enhancing the formation of cluster DNA damage.^{95–97} LEE were found to be the main radiolytic species responsible for this damage enhancement, via the formation of more destructive transient negative ions, when the drug is bound to DNA.⁹⁴

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FIG. 1.

Exposure-response curves for the formation of crosslinks (CL), single and double strand breaks (SSB and DSB), and loss of supercoiled DNA in 5 ML films induced by 9.6 eV electrons.

FIG. 2. Effective yields of crosslink and linear (DSB) configurations of DNA induced by 1.6–19.6 eV electron impact on DNA films of 5 ML thickness.

FIG. 3. Effective yields of SSB and loss of supercoiled configuration of DNA induced by 1.6–19.6 eV electron impact on DNA films of 5 ML thickness. The dashed lines represent simulated background yields.

FIG. 4.

Comparison of DNA damages induced by 1.6–20 eV electron impact on DNA films composed of (A) plasmid-diaminopropane cation complexes (pUC21-Dap²⁺). Reprinted with permission from J. Chem. Phys. **139**, 055104 (2013). Copyright 2013 AIP Publishing LLC; (B) oligonucleotides GCAT integrating the four DNA bases;⁵⁴ (C) linear DNA composed of 40 base pairs and pGEM-3Zf(-) plasmid DNA;⁵⁵ (D) pGEM-3Zf(-) plasmid DNA;³⁷ (E) pGEM-3Zf(-) plasmid DNA from present study, (F) p14 plasmid DNA³⁹ and (G) pQE3 plasmid DNA.⁴¹ The detected damages were (A) loss of supercoiled, (B) fragment products identified in the legend, (C) H⁻ESD yields and (D) to (G) SSB.

SCHEME 1.

Decay channels of transient negative ions of DNA bases formed at an initial electron energy of E_0 . Pathways 1–3 represent the elastic ($E = E_0$), dissociative electron attachment (DEA), and electronically inelastic ($E \ll E_0$) channels, respectively. e^-_c represents the situation when the electron is 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 by electron transfer dissociates, leading to C–O bond scission.

TABLE I

Effective yields (in 10^{-15} electron $^{-1}$ molecule $^{-1}$) of crosslinks (CL), single and double strand breaks (SSB, DSB), and loss of initial super-coiled DNA induced by 1.6–19.6 eV electrons in 5 ML thick films. The error bars are the standard deviation of the slope at zero fluence.

Energy (eV)	Configuration			
	Crosslinks	Linear (DSB)	Circular (SSB)	Supercoiled
1.6	4.1 ± 0.6	BDL ^a	28.5 ± 4.0	-34.8 ± 1.9
3.6	9.0 ± 1.0	1.1 ± 0.3	42.9 ± 7.3	-57.5 ± 7.3
4.6	7.5 ± 1.5	2.5 ± 0.5	65.9 ± 9.4	-85.8 ± 13.6
5.6	9.6 ± 1.1	4.3 ± 0.7	38.2 ± 4.2	-42.7 ± 5.8
6.6	8.3 ± 1.1	4.3 ± 0.5	45.0 ± 5.2	-51.2 ± 7.3
7.6	9.4 ± 1.4	2.3 ± 0.4	72.2 ± 10.9	-82.6 ± 13.6
9.6	9.7 ± 1.2	3.8 ± 0.5	92.1 ± 3.1	-118.2 ± 15.7
11.6	9.7 ± 1.2	2.5 ± 0.4	54.4 ± 7.3	-81.6 ± 8.4
13.6	7.7 ± 0.8	2.1 ± 0.4	32.4 ± 6.3	-42.9 ± 9.4
15.6	7.6 ± 1.5	2.8 ± 0.3	37.7 ± 2.1	-49.2 ± 4.2
17.6	8.5 ± 1.4	3.2 ± 0.3	45.0 ± 4.2	-62.8 ± 5.2
19.6	6.0 ± 0.8	3.8 ± 0.4	49.2 ± 4.2	-70.1 ± 6.3

^aBDL: below detection limit.

TABLE II

Experiments on LEE-induced damage to DNA performed by different laboratories. The alphabet in the first column refers to the yield functions shown in Figure 4. (OT: Onset of electron transmission; RT: room temperature; HPLC: high-performance liquid chromatography; ESD: electron-stimulated desorption; E: Electrophoresis; No: Not recorded.).

	Film			Energy calibration	Current density (nA/mm ²), fluence (electrons/DNA)	Fluence response curve	Method of analysis
	Preparation	Thickness	Substrate				
A ³⁸	Dap ²⁺ ; intercalated	10 ML	Graphite	No	0.6, 18	No	E
B ⁵⁴	Spin-coating	5 ML	Tantalum	No	4.1, 2.5	No	HPLC
C ⁵⁵	Dried in N ₂ at RT	1.5, 3 ML	Tantalum	OT	0.4	No	ESD
D ³⁷	Lyophilization	5 ML	Tantalum	No	3,6,0 ^d	Yes	E
E	Lyophilization	5 ML	Tantalum	OT	0.5,0 ^d	Yes	E
F ³⁹	Dried at RT	>50 ML	Tantalum	No	N, ~410	No	E
G ⁴¹	Dried at RT	5ML(estimated)	Tantalum	No	0.2, 6.8	No	E

^dExtrapolated to zero fluence from exposure-response curve.

TABLE III

Most intense core-excited resonances of the bases and their decay channels below 16 eV. The DEA results are obtained from Refs. 81 and 82. The resonances are classified as belonging to one of three energy range I (5–6 eV), II (8.5–11 eV), and III (13–16 eV).

Base/oligo	Resonance energy (eV)	Decay channels
Thymine ⁸	5 (I)	$1^3A' (\pi_2 \rightarrow \pi_3^*) + 1^3A'' (n_2 \rightarrow \pi_3^*)$
	8.5 (II)	$2^3A' (\pi_1 \rightarrow \pi_3^*) + 2^1A' (\pi_2 \rightarrow \pi_3^*)$
	8.5 (II)	$3^3A', 2^3A'', 4^3A', 3^3A'', 3^1A'$
	9 (II)	$5^3A' (\pi_0 \rightarrow \pi_3^*) + 5^1A' (\pi_1 \rightarrow \pi_4^*)$
	9.3 (II), 15.9 (III)	DEA (H ⁻)
	9.9 (II), 13.9 (III)	DEA (CN ⁻)
	9.8 (II)	DEA (OH ⁻)
	12.7 (III)	DEA (OCN ⁻)
	10.7 (II)	DEA (CH ₂ ⁻)
Cytosine ⁹	6 (I)	$1^3A' (\pi \rightarrow \pi^*)$
	10 (II)	$3^{3,1}A' (\pi \rightarrow \pi^*)$
	11 (II)	$4^1A' (\pi \rightarrow \pi^*)$
	12 (II)	$5^1A' (\pi \rightarrow \pi^*)$
	12 (II)	$6^1A' (\pi \rightarrow \pi^*)$
	8.5 (II), 10.3 (II)	DEA (H ⁻)
	8.9 (II)	DEA (CN ⁻)
	8.8 (II)	DEA (O ⁻)
	11 (II)	Ionization
Adenine ¹⁰	10 (II)	$n \rightarrow \pi^*$
	10 (II)	$\pi \rightarrow \sigma^*$
	10 (II)	$\pi \rightarrow \pi^*$
	9.5 (II), 14.3 (III)	DEA (H ⁻)
	9.8 (II), 15.3 (III)	DEA (CN ⁻)
Guanine	10.6 (II), 15.0 (III)	DEA (H ⁻)
	9.0 (II), 13.9 (III)	DEA (CN ⁻)
	9.0 (II), 13.2 (III)	DEA (O ⁻)
	8.7 (II), 12.7 (III)	DEA (OH ⁻)
GCAT ⁸²	9.2 (II)	DEA(H ⁻)
	9.0 (II), 11.3 (II)	DEA (O ⁻ /NH ₂ ⁻)
	9.1 (II)	DEA (OH ⁻)