Influence of organic ions on DNA damage induced by 1 eV to 60 keV electrons

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Abstract

We report the results of a study on the influence of organic salts on the induction of single strand breaks (SSBs) and double strand breaks (DSBs) in DNA by electrons of 1 eV to 60 keV. Plasmid DNA films are prepared with two different concentrations of organic salts, by varying the amount of the TE buffer (Tris-HCl and EDTA) in the films with ratio of 1:1 and 6:1 Tris ions to DNA nucleotide. The films are bombarded with electrons of 1, 10, 100, and 60 000 eV under vacuum. The damage to the 3197 base-pair plasmid is analyzed *ex vacuo* by agarose gel electrophoresis. The highest yields are reached at 100 eV and the lowest ones at 60 keV. The ratios of SSB to DSB are surprisingly low at 10 eV (~4.3) at both salt concentrations, and comparable to the ratios measured with 100 eV electrons. At all characteristic electron energies, the yields of SSB and DSB are found to be higher for the DNA having the lowest salt concentration. However, the organic salts are more efficient at protecting DNA against the damage induced by 1 and 10 eV electrons. DNA damage and protection by organic ions are discussed in terms of mechanisms operative at each electron energy. It is suggested that these ions create additional electric fields within the groove of DNA, which modify the resonance parameter of 1 and 10 eV electrons, namely, by reducing the electron capture cross-section of basic DNA units and the lifetime of corresponding transient anions. An interstrand electron transfer mechanism is proposed to explain the low ratios for the yields of SSB to those of DSB produced by 10 eV electrons.

I. INTRODUCTION

In recent years, our knowledge of the interaction between DNA and low energy electrons (LEEs) has grown considerably, and we now understand some of the fundamental mechanisms of LEE-induced DNA damage. This damage occurs on a molecular scale,¹ where the fragmentation is often localized on the individual DNA subunits (i.e., DNA bases,²⁻⁵ deoxyribose,^{3,6} phosphate,^{4,7} or the surrounding water molecules^{8,9}). Below 15 eV, fragmentation occurs mainly via the formation of transient anions, which decay into the dissociative electron attachment (DEA) channel or by auto-ionization; this latter channel can leave a subunit in a repulsive state, thus causing fragmentation. At higher energy, such

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excited states result principally from direct scattering.^{1,10} All of these processes can irreversibly modify DNA^{1,10} producing base and sugar modifications, base release, single strand breaks (SSBs), and clustered lesions, which include a combination of two or more single modifications, e.g., double strand breaks (DSBs), tandem lesions, and crosslinks.¹¹ At the experimental level, our knowledge of LEE-DNA interactions was derived from a multitude of experiments of increasing target complexity, ranging from gaseous DNA basic units or their analogs^{5-7,12} to films of plasmid DNA in vacuum.¹³

Although the dry film experiments of pure DNA in vacuum were essential to unveil basic mechanisms of damage, they clearly do not correspond to cellular conditions. We therefore initiated a series of systematic investigations to probe DNA with LEE under conditions that are closer to those found in living cells. We first showed that the presence of additional water around DNA modifies the transient negative ion manifold and corresponding decay channels.¹⁴ Then, in experiments performed at atmospheric pressure with a humidity level of 65%, total damage to DNA induced by LEE was measured under conditions much closer to the hydrated and aerobic environment of the cell.¹⁵ The indirect effect of LEE irradiation (i.e., damage to DNA via reactive species created by LEE irradiation with molecules surrounding DNA) was found to contribute to 60% of the damage.¹⁵ More recently, Dumont *et al.*¹⁶ studied the influence of organic ions on the mechanisms of SSB induction by 10 eV electrons. Tris and EDTA were incorporated at various concentrations within DNA films of different thicknesses. The yield of SSB was found to decrease dramatically as a function of the number of organic ions per nucleotide. As few as two organic ions/nucleotides were sufficient to decrease the yield of SSB by 70%. This effect could be explained by the protection of DNA against LEE-induced damage by the presence of the organic ions, once the effect of increasing multiple inelastic electron scattering with film thickness was removed.

Ions are an important constituent of cells; they play a role in the stabilization of the B-DNA conformation *in vitro* by their interaction with the major and minor grooves, as well as the negatively charged phosphate.¹⁷ Apart from these characteristics, the organic ions investigated by Dumont *et al.*¹⁶ are of special interest, since their concentration (10 mM Tris with 1 mM EDTA) constitutes the standard buffer solution for many *in vitro* experiments with DNA.^{1,11} Furthermore, as shown in Fig. 1, the Tris molecule possesses an amine group (NH₂), while EDTA molecule contains four carboxylic acid groups. Owing to their functional groups, it is likely that these organic ions can mimic some aspects of the protein structure, such as those of the histone proteins, which are intimately associated with the DNA of eukaryotic cells.¹⁷ Experiments with these molecules can therefore provide some insight into the effect of proteins on LEE-induced DNA damage.

In the present study, we extend the experiments of Dumont *et al.*¹⁶ to the energy range of 1 eV to 60 keV and measure both SSB and DSB induced by electron impact on thin films of plasmid DNA under vacuum. We chose to work with two characteristic salt concentrations: one organic ion/nucleotide, which is necessary to neutralize the charge on the phosphate group of the backbone and hence corresponds to minimal salt concentration; and six organic ions/nucleotides, which correspond to a high level of protection at 10 eV. The films were bombarded at electron energies of 1, 10, 100, and 60 000 eV, which are characteristic of

specific phenomena. At 1 eV, LEE can damage DNA only via shape resonances leading to the formation of a dissociative transient anion¹⁸ (i.e., DEA). DNA damage via transient anion formation is maximum at 10 eV, where core-excited resonances break bonds by decaying into DEA or autoionization leading to excitation of dissociative electronic states.^{1,13} Around 100 eV, electron scattering within DNA films produces maximum ionization and hence the largest density of LEE within the film.¹⁰ Finally, 60 keV electrons can be taken as a representative of the fast electrons, such as Compton electrons, created during patient treatment with photons of 1–25 MeV.¹⁹ The yields of SSB and DSB measured at these different energies and salt concentrations are reported in this article. They are discussed in terms of LEE interactions with DNA, which differ at each characteristic energy.

II. METHODS AND EXPERIMENTS

A. Preparation of plasmid DNA

pGEM-3Zf(–) plasmid DNA (3197 base pairs, Promega) was extracted from *E. coli* DH5 α , purified with QIAfilter Plasmid Giga Kit (Qiagen).²⁰ Agarose gel electrophoresis analysis showed that 95% of the purified plasmid DNA was in the supercoiled form and the rest was in the concatemeric (1%) and nicked circular form (4%). The absolute amount of plasmid DNA was determined by measuring its UV absorption at 260 nm.²¹ The relative amount of proteins remaining in the plasmid was obtained from the ratio of 1.98 of the UV absorption of DNA from 260 to 280 nm; this ratio corresponds to less than 15% by weight of proteins remaining with DNA.^{22–24}

The volume of TE (Tris-HCl and EDTA) was adjusted with respect to the quantity of DNA to obtain a final molar ratio of 6:1 of salt to DNA nucleotide. Alternatively, the buffer TE was removed by a Sephadex G-50 (Pharmacia) column. The efficiency of the Sephadex G-50 column to remove the small molecule is about 99.9% before saturation.²⁵ Thus, the Sephadex column removes the vast majority of the TE ions not acting as counterions for the DNA phosphate group. The minimum salt:nucleotide ratio obtainable is 1:1. However, we cannot exclude the possibility of some trapping of additional organic ions within the groove of DNA. Hence, we consider that in our DNA sample, after purification by the Sephadex column, the salt ratio lies between 1:1 and 2:1, although it is probably closer to 1:1 (i.e., 1:1 is the ratio number of the structural salt per nucleotide).¹⁷ For simplicity, we refer to this ratio as 1:1 in the following text. Considering the stoichiometric structure of Tris and EDTA (Fig. 1), the positively charged amide group of Tris most likely interacts with DNA and could protect the molecule. The interaction of EDTA with DNA should be less important owing to its four negative charges. In addition, the amount of EDTA added in the present experiment is ten times less than Tris. Thus, in the following text, we mainly discuss the effect of organic salts in term of the Tris-DNA interaction.

B. LEE irradiation

Under dry nitrogen atmosphere, 245 ng of DNA with or without 353 ng of TE in 10 μ l dd H₂O was deposited on a chemically clean tantalum foil (Goodfellow, 99.9% purity, 25 μ m thickness). The deposit was frozen at –70 °C for 5 min, and then lyophilized with a hydrocarbon-free sorption pump under a pressure of 1 mTorr for 2 h. The lyophilized pure

DNA formed a film with a diameter of 3.5 ± 0.5 mm and thickness of 15 nm (5 ML), assuming a density of DNA of 1.7 g/cm^3 , and a monolayer (ML) corresponding to a thickness of 3 nm.^{26,27} With the added salt in ratio of 6 per nucleotide, the film had a final thickness of 9 ML. The samples were directly transferred to an ultrahigh vacuum chamber (UHV). After 12 h evacuation, the background pressure reached 10^{-8} Torr. Afterward, the DNA was exposed at room temperature to a 1.5 nA electron beam. The incident electron energy was set at 1 ± 0.5 , 10 ± 0.5 , and 100 ± 0.5 eV, respectively, for irradiation times varying from 5 s up to 2 min at each energy. Since the dose (i.e., the amount of energy deposited per mass) cannot be easily evaluated in thin film experiments with electrons, the time-response of the yields was expressed in terms of the fluence (i.e., the total number of electrons hitting the target). To obtain the fluence-response curve at each salt concentration and electron energy, 36 samples had to be irradiated with six identical experiments for a given fluence. For each fluence, a control sample was deposited on the tantalum foil, lyophilized, kept under UHV conditions, and recovered, but was not irradiated in order to monitor the change in supercoiled DNA due to manipulation and to record the zero fluence data point in the exposure curve.

C. 60 keV electron irradiation

5 μl aliquots of DNA solutions with salt ratio of 1:1 and 6:1 per nucleotide were deposited on a 1-mm-thick gold foil (99.99%, Laboratoire MAT). The samples were dried in a glove box at ambient temperature, at a relative humidity of 10%. This procedure produced films of 2900 nm thickness estimated from the density of DNA of 1.7 g cm^{-3} .^{26,27} This thickness was chosen to absorb sufficient energy from the electron beam, while avoiding significant damage from secondary electrons emitted from the metal substrate.

Afterward, the samples were transferred to the transmission electron microscopy (TEM) (H-7100 Hitachi) chamber, where they were irradiated (or not for the controlled samples) by a 60 keV electron beam with a current of 15 μA for periods varying from 5 to 30 s. Data were recorded at six different fluencies (including zero fluence) under identical experimental conditions; each data point was the average of three experiments. The incident electron fluence of the TEM was measured with a radiochromatic dosimetry film as described previously.²⁸ Taking into account the area of the electron beam of 4.6 mm^2 , the incident electron flux was determined to be $2.9 \times 10^{13} \text{ electrons s}^{-1} \text{ cm}^{-2}$.

D. Analysis of plasmid DNA by agarose gel electrophoresis

Considering the four electron energies at which the targets were bombarded, a total of 288 samples were analyzed by the following procedure. After irradiation, the tantalum or gold foil was removed from the vacuum chamber and the sample was immediately dissolved in 10 mM Tris-HCl, pH 7.5. The recovery of DNA was approximately 98% and 90% for the tantalum and gold, respectively. The different forms of DNA were separated by 1% neutral agarose gel electrophoresis run in TAE buffer (40 mM Tris acetate, 1 mM EDTA, pH 8.0) at 100 V for 7 min and 75 V for 90 min. Both the gel and the DNA samples were prestained by SYBR Green I (Molecular Probes), 10 000X for gel and 100X for samples, respectively. After electrophoresis, gels were scanned with the STORM860 system (Molecular Dynamics) using the blue fluorescence mode at an excitation wavelength of 430 nm. The

percentage of each form was obtained from Image Quant analysis. These values were corrected for the weaker binding of SYBR Green I to the supercoiled form of DNA compared to the nicked circular (SSB) and linear (DSB) configurations.²⁹ To obtain this correction factor, 100 ng of supercoiled DNA and 100 ng of linear DNA were deposited in two different wells of an electrophoresis gel. The linear fragments of DNA were obtained by cutting the plasmid with the restriction enzyme EcoRI, which linearized the plasmid. After determining the intensity of each band, the area under the peak of linear DNA was divided by the area under the peak of supercoiled DNA to give a correction factor of 1.7.²⁹

III. RESULTS

The fluence-response curve for the induction of SSB and DSB by 100 eV electrons with salt ratio of 1:1 and 6:1 is shown as an example in Fig. 2. The number of breaks increases with the incident electron fluence and saturates eventually with time of exposure. Two major factors that affect the fluence-response curve are the depletion of available targets and film charging. For this reason, yields are measured from the slope of the fluence-response curve at low fluencies where these factors are small and have little effect on the data. Furthermore, in this linear region, the yields result from the interaction of a single LEE causing the measured type of damage.³⁰ If no charging occurs, the yield of SSB at higher fluencies becomes an exponentially saturating function due to the depletion of the initial number of source molecules (i.e., supercoiled DNA).³⁰ The situation is different for the yields of DSB; as seen in Fig. 2, the yields of DSB saturate much more slowly than the yield of SSB. This phenomenon can be explained by considering that the circular form is also an initial source target for the production of DSB, thus, the source for DSB is not depleted as fast with increasing fluence.³¹

From fluence-response curves recorded at each energy, the yields for electrons of 1 eV to 60 keV were obtained and are listed in Table I. They are expressed as the number of SSB or DSB per incident electron and DNA molecule for the different electron energies and salt ratios (1:1 and 6:1). They are therefore independent of the film thickness for a linear absorption of energy with electron penetration distance in the film. For 60 keV electrons, this is essentially the case, since the film thickness is considerably smaller than the penetration depth required for absorbing all the electron energy. For 1, 10, and 100 eV electrons, this penetration depth is of the order of the film thickness, so that the effect of elastic and inelastic multiple scattering slightly lowers the measured yields.³² Thus, although the yields at 1, 10, and 100 eV may appear high compared to those recorded at 60 keV, they still represent lower limits. For comparison, the results of two other papers also obtained at 10 eV with 1:1 and 6:1 salt to nucleotide ratios of the same organic ions (i.e., same buffer as ours) are also listed in Table I. In our experiments and some of those of Dumont *et al.*,¹⁶ the films contained the same amount of DNA (245 ng). In this case, the presence of salt molecules increased the film thickness from 5 ML with ratio of 1:1 to 9 ML with the ratio of 6:1.

Dumont *et al.*¹⁶ discussed the effect of film thickness on the yields of strand breaks. On one hand, the increase of electron multiple elastic scattering with thickness can increase the path length of electrons in the film and consequently the yield of strand breaks.³² However, the

loss of electron energy due to inelastic collisions in the film has the opposite effect.^{16,32,33} To estimate the significance of multiple scattering, Dumont *et al.*¹⁶ measured the yield of strand breaks as a function of the number of ions present near the DNA at constant and variable film thicknesses. The yield of strand breaks in samples with constant DNA content and increasing film thickness exhibited the combined effect of multiple scattering, and the presence of organic ions on DNA damage, while the yields recorded at constant film thickness were only influenced by the ions. Thus, we can extrapolate two yields from the data of Dumont *et al.*¹⁶ By comparison of these two sets of data, we find that, in our experiment, where the amount of DNA was kept constant with different salt ratios, there is a negligible variation in the yield of strand breaks due to the change of film thickness.

The other yield of SSB at 10 eV shown in Table I was calculated from the exposure curves reported by Panajotovic *et al.*³⁰ A factor of 2 and 3 exists between our data and those of Dumont *et al.*¹⁶ and Panajotovic *et al.*³⁰ Within a given series of measurements with the same batch of DNA and experimental conditions, the standard deviation, as quoted in Table I, is not too large; but, comparison of absolute values includes many sources of errors, which arise from variations in the large number of parameters involved in these experiments (e.g., from experimental conditions which are difficult to control). We therefore consider that the yields quoted in Table I represent a fairly good agreement.

What is easier to compare from one experiment to another is the ratio of SSB to DSB, since in this case the error on absolute yields is considerably reduced. Table II lists the ratios obtained by various authors for electron and photon irradiation of *dry* DNA at different energies. The salt concentration is specified when known. The numbers in parenthesis represent the lower and upper limits of these ratios calculated from the standard deviations of respective yields, when available. Photoelectrons emitted from a tantalum metal surface were the electron source in the experiment of Cai *et al.*²⁹ The energy spectrum had a distribution ranging essentially from 0 to 20 eV. The ratio has been obtained by correcting for electrons below 5 eV, which do not produce DSB.¹⁸ Other experiments with electrons were performed with a direct beam source.^{10,34,30,33} From the same experiment, Cai *et al.*²⁹ obtained a ratio of 11.4 for 1.5 keV photons in excellent agreement with the calculation of Fulford *et al.*³⁵ The results of Le Sech *et al.*³⁶ were recorded above (SSB/DSB=11) and below (SSB/DSB=13) the energy of LIII inner shell absorption of Pt, which was attached to the DNA.

The yields obtained in the present experiment are plotted as a function of electron energy in Fig. 3. The yields of SSB and DSB in DNA films containing a salt ratio of 1:1 (I) are considerably larger than those of DNA films with salt ratio of 6:1 (II). The highest yields are reached at 100 eV. From 1 to 100 eV, the smallest decrease of damage with increasing salt concentration lies at 1 eV. However, in terms of percentage (bottom of Table I), the decrease in damage is about the same at 1 and 10 eV, within experimental error, whereas at 100 eV, the protection imparted by the organic salt is reduced. The opposite effect is observed for DSB: at 10 eV, damage is reduced by 29%, a value which increases to 33% at 100 eV. Comparing the percentages at the lowest energies with those found at 100 eV and 60 keV (Table I), we find that Tris offers the highest protection to SSB induced by 1 and 10 eV electrons.

IV. DISCUSSION

The results of Fig. 3 and Table I clearly show that the damage induced by electrons depends on their energies and on the presence of salt around the DNA. In this section, we discuss these differences in terms of the known mechanisms of DNA damage at each energy.

A. Damage induced by 1 eV electron

At 1 eV, below the electronic excitation threshold, only shape resonances can be involved in the bond breaking process.^{1,18} In DNA, such electron resonances usually arise from the capture of an incident electron in an empty orbital of a base or the phosphate group for a time, which is greater than the usual scattering time.¹ When the transient anion state is dissociative and the resonance lifetime is greater than about half a vibration period of the anion, the latter can dissociate (i.e., DEA). Zheng *et al.*⁶² showed that LEE could break the backbone of DNA by DEA on the phosphate group, leading to the rupture of the CO bond.³⁷ The electron is either first directly captured by the phosphate group or captured by a base and then transferred to the CO bond of the phosphate group.³⁸ It is therefore possible that the resonance parameters of the transient anions involved in the rupture of a DNA strand, via these two pathways, could be modified by the presence of organic ions within the grooves of DNA.

It is well established that both the stable helix-helix distance and the strength of the attraction in DNA are strongly dependent on the salt concentration and ion size.³⁹ With the increase of the salt concentration, the helix-helix attraction becomes stronger indicating that the electric fields within the molecule are modified.³⁹ The DNA becomes more stable as the helix-helix separation distance becomes smaller and transient anions formed by electron attachment are expected to be affected by the modification of internal electric fields. The cross-section for DEA to a base or the phosphate group depends on the electron capture probability and the survival probability of the anion against autodetachment of the electron. In the case of electron transfer, the capture probability depends on the direct capture cross-section by the base and the electron transfer probability from the base to the phosphate group at 1 eV.

In DNA, any change in the environment of a base or a phosphate unit is bound to influence the magnitude of the DEA cross-section to the basic unit, most effectively, by modifying the autodetachment probability of the captured electron. For example, modification of an electric field close to a molecule can considerably alter the DEA cross-section.^{40,41} This has been verified by changing the distance between a molecule undergoing DEA and its metal substrate by intercalating an inert dielectric layer between them. At large distances (~ 1 nm), the electric field induced by the polarization field of the transient anion in the dielectric and in the metal increases the DEA cross-section principally by reducing autoionization.^{40,42} At shorter distances from the metal surface (~ 1 nm), the electric field is sufficiently high to decrease the capture cross-section and reduce the average lifetime by increasing the electron transfer to the metal.^{40,42} Considering the sensitivity of DEA to the modification of a near electric field, it is very likely that changes of electric fields⁴² within the DNA caused by the addition of cations at specific sites would modify the magnitude of the DEA process responsible for the rupture of the CO bond of the backbone. It is therefore highly probable

that changes in the internal charge distribution by Tris cations within the DNA are involved in the observed protection by organic ions at 1 eV.

One could also envisage quenching of the transient anion state by proton or electron transfer caused by the presence of the organic cations. Proton transfer can be as fast as the anion's lifetime,⁴³ and could therefore suppress the anion state before it dissociates or before it transfers the electron from the base to the phosphate group. Nothing is known about proton interaction with *transient anions*, but reversible and irreversible proton transfer to *stable anions* has been observed and reported in the literature.^{44–49} On the other hand, electron transfer could be modified by Tris cations. When inserted within the groove of DNA, such an ion creates an attractive potential to electrons with its positively charged NH_3^+ group. If Tris is sufficiently close to a base, another electron transfer channel becomes available, thus reducing transfer to the phosphate group and hence strand breaks. Alternatively, if the attractive potential is felt by the phosphate group, electron transfer to Tris would directly reduce breaking of the CO bond via DEA. Thus, quenching of the transient anion by proton or electron could also at least partially account for protection by organic ions.

B. Damage induced by 10 eV electrons

At 10 eV, electrons break DNA strands essentially via core-excited resonances, i.e., the formation of a transient anion consisting of the incoming electron trapped by the positive electron affinity of an electronically excited state of a basic unit of DNA.^{1,10} When created on the phosphate unit of DNA, these two-electron one-hole states, when dissociative in the Franck–Condon region, could lead to the rupture of the CO bond in the backbone.^{37,38} At this energy, the incident electron can also be captured by a base and subsequently transfer to the dissociative anion states of the phosphate group. In fact, even at this higher energy, the transfer of electrons from a base to the phosphate group appears to be involved in breaking DNA strands.³⁸ However, at 10 eV, the electron may lose a considerable amount of energy to electronically excite a base before transferring. In this case, the electron's energy lies below 0–5 eV. The exact energy depends on that of the electronically excited state and polarization of the local vibrational modes by the added charge. So, the electron transfer process and its magnitude may be similar at 10 eV to the ones described for 1 eV electrons. The mechanisms involved in the organic ion protection described previously for 1 eV electrons could therefore also be operative at 10 eV.

At 10 eV, the base transient anion can also dissociate (DEA), producing a neutral moiety and an anionic fragment.⁴ The additional electron on the anionic fragment could also transfer to the PO_4 unit, if it is only weakly bound. This type of transfer could provide an additional mechanism leading to the formation of a transient PO_4^- anion dissociating the CO bond and thus producing a SSB. In this case, proton transfer from the Tris molecule may play a role in the protection of DNA by organic ions if the anionic fragment is a dehydrogenated anionic base (i.e., the added proton would restore the original base). Jena *et al.*⁵⁰ showed from the density functional theory calculations that this type of mechanism in certain amino acids, such as cysteine and tyrosine, can repair damage to the bases.

The intramolecular electron transfer mechanism described in this section to explain the formation of SSB may also be helpful in trying to account for the surprisingly low ratios of

SSB to DSB observed at 10 eV (i.e., first to third line in Table II). As suggested by Orlando *et al.*,³⁴ two successive breaks induced by a single LEE could provide an explanation. The cross-section for a 10 eV electron to break one bond in the backbone of a DNA molecule made of 3197 base pairs is about 10^{-15} cm^2 .³⁰ This value translates into a cross-section of about $3 \times 10^{-18} \text{ cm}^2$ for such an electron to hit a group of ten base pairs. Thus, the probability of a 10 eV electron to make two successive breaks within the distance of ten base pairs (i.e., a DSB) along its path is much too low to account for the observed ratios. Another possibility is reactive scattering. In this case, DEA to the phosphate group would break one bond and create a neutral or anion radical that could traverse to the other strand and cause a bond breaking reaction. The probability of such reactions has been tested in specific experiments, where a hydrocarbon film was doped with O_2 molecules.⁴¹ DEA to O_2 created O^- radicals that scattered reactively with hydrocarbon molecules, causing OH^- to be expelled from the film. The amount of OH^- produced was about two orders of magnitude lower than O^- desorption. Therefore, it appears unlikely that reactive scattering could lead to the observed ratios of SSB to DSB.

The efficiency of intrastrand electron transfer to cause SSB in DNA is well established both theoretically⁵¹ and experimentally.^{18,38,52} If interstrand electron transfer occurs with a similar efficiency, it could perhaps explain the low ratios listed in Table II for 10 eV electrons. However, it is not known if such a process occurs and how it could lead to DSB. In the simplest case, the transient anion formed on the phosphate group decays by autoionization, leaving the CO unit in the backbone in an electronic excited state that dissociates breaking the CO bond. Simultaneously, the departing electron transfers to the opposite strand and forms a transient anion of the phosphate group causing a CO bond to be broken on the other chain by DEA. Such breaks, which are close to each other, would be difficult to repair. More theoretical input is needed to test the validity of this hypothesis or other plausible mechanisms.

C. Damage induced by 100 eV electrons

At 100 eV, many nonresonant mechanisms including neutral dissociations, excitation, and single and multiple ionizations in DNA may lead to bond cleavage. These include transitions to excited states of the neutral molecule [i.e., for a molecule $\text{RH}:\text{e}^-(E_0) + \text{RH} \rightarrow (\text{RH})^* + \text{e}^-$ ($E < E_0$)] which, if dissociative, can produce at least two neutral radicals, $\text{R}^\bullet + \text{H}^\bullet$ or a cation-anion pair, $\text{R}^+ + \text{H}^-$, or $\text{R}^- + \text{H}^+$ screened by the polarization they induce in the film. Similarly, single ionization with or without dissociation may lead to formation of reactive transients via $\text{e}^- + \text{RH} \rightarrow (\text{RH})^{+} + 2\text{e}^-$, or $\text{e}^- + \text{RH} \rightarrow (\text{RH})^{3+} + 2\text{e}^-$ followed by $(\text{RH})^{*+} \rightarrow \text{R}^\bullet + \text{H}^+$, or $\text{R}^{+} + \text{H}^\bullet$. Owing to the steeper potential energy surfaces at higher electron energies, we expect the Franck-Condon overlap integral between the nuclear wave functions of ground state RH and RH^* to be smaller than the overlap integral of the wave functions of RH and RH^{+*} . We therefore expect a larger amount of $\text{R}^\bullet + \text{H}^+$ and $\text{R}^{+} + \text{H}^\bullet$ species to be produced than the pair $\text{R}^\bullet + \text{H}^\bullet$. Furthermore, based on the chemical aspect of RH heterolysis, $\text{R}^{+} + \text{H}^-$ pair formation via RH^* seems less likely than the formation of $\text{R}^- + \text{H}^+$ pairs. Once thermalized, these species should have different reactivity. More damage is expected to be induced from $\text{R}^\bullet$, R^+ , H^+ , and $\text{H}^\bullet$ than from R^- and H^- . The cross-section for the sum of ionization and fragmentation channels is highest at 60–100 eV for most organic molecules and lies in the

10^{-15} – 10^{-16} cm² range.^{53,54} It is therefore not surprising to find that at 100 eV, the SSB or DSB yield intensities possess the highest magnitude. However, considering that the total cross-section for dissociation via resonance electron capture at 10 eV lies in the 10^{-18} cm² range in biological condensed matter,⁵⁵ it is surprising to find a magnitude for the yields of SSB and DSB at 10 eV similar to that at 100 eV. This suggests that in plasmid DNA films, the total contribution to the SSBs or DSBs yields from all nonresonant mechanisms, available to incident 100 eV electrons (including the secondary electrons created by them), is similar to the total contribution of all resonant mechanisms by which 10 eV electrons can initiate damage to DNA. This result illustrates the efficiency of LEE, and particularly those at 10 eV, to damage DNA via the formation of transient anions as compared to other processes.

D. Damage induced by 60 keV electrons

Strand breaks produced by 60 keV electrons (Table I) were recorded to illustrate the different efficiencies of low and high-energy electrons. 60 keV electrons are representative of high-energy particles.¹⁹ Along their path in biological matter, they lose their energy by an electromagnetic type of interaction: biological molecules absorb quanta of energy (virtual photons) from swift electrons.⁵⁶ Thus, fast charged particles of low linear energy transfer (LET) are expected to behave like photons and produce similar yields of damage in DNA. According to oscillator strengths in organic material, about 20% of the absorption of the energy of 60 keV electrons leads to the formation of electronically and vibrationally excited species, whereas 80% of that energy causes ionization.⁵⁴ The latter process creates LEE, whose distribution maximizes around 9 and 10 eV.⁵⁷ The yields of SSB at 10 eV given in Table I are at least an order of magnitude higher than those recorded at 60 keV. The ratios of SSB produced at 100 eV relative to those produced by 60 keV electrons are 21 and 17, for salt concentrations of 1:1 and 6:1, respectively. These ratios can be considered as lower limits, since 1–100 eV electrons deposit essentially all of their energies in a 5 ML DNA film, whereas 60 keV electrons deposit only a small portion of their energy in 3–5 μ m films. Thus, the mechanisms operative at high energy are much less efficient than those acting at 1–100 eV to damage DNA. Furthermore, high-energy mechanisms are the least affected by the presence of organic ions in our films. We can therefore speculate that the reactivity of species, other than that of the LEE created by the 60 keV beam, is less affected by the presence of organic ions.

E. Ratios of the yields of SSB to DSB

The results between 10 and 100 eV for the ratios of the yields of SSB/DSB at different salt concentrations are shown in Table II. Based on the error limits, it is difficult to reach any conclusion from comparison of a specific ratio to another. What is more significant, however, is that the mean ratio from each experiment lies between 3.9 and 4.6 at 10 eV and within the range of 3.8 and 5.1 at 100 eV, with an average of 4.2 and 4.3, respectively. Thus, both 10 and 100 eV electrons are extremely efficient in causing DSB as compared to SSB. As seen in Table II, photons of low energy (i.e., 8–25 eV) give yield ratios varying from 20 to 12, respectively. According to the synchrotron measurements of Michael *et al.*,⁵⁸ at 10 eV, where the ionization efficiency is low for both electrons and photons, the latter give a ratio of about 18 (i.e., 4.6 larger than the ratio for 10 eV electrons). These comparisons are

significant and indicate that, while radicals produced by photons may efficiently break single strands, their ability to produce a DSB is relatively small. As the energy of photons increases, the medium is further ionized and much more LEE and ions are produced. Since the SSB/DSB ratio decreases with increasing photon energy beyond 25 eV, we may conclude that the density of highly reactive species increases (i.e., the increasing number of radicals, ions, and LEE produced within the diameter of DNA leads to a greater potential to create DSB). The same ratio as for 10 eV photons is found for ^{60}Co (1 MeV) γ radiation,⁵⁹ which produces essentially fast Compton electrons.¹⁹ As previously stated, such electrons act as electromagnetic radiation producing ions and LEE with a most probable energy loss of about 22 eV.⁵⁴ It is therefore not surprising to find ratios similar to those of 8–25 eV photons. Similarly, the ratio is still fairly high for 16 keV photons (SSB/DSB=13), but becomes lower when more ionizations and LEE are created by exciting an inner shell of an atom close to the DNA³⁶; in the case given in Table II the ratio is lowered from 13 to 11 by the emission from Pt of L_{III} Auger electrons.³⁵ At 1.5 keV, the SSB/DSB ratio for photons is still high (Cai *et al.*²⁹ and Fulford *et al.*³⁵ in Table II), but as the energy decreases the LET increases causing a decrease in the ratios. Evidence for this trend is shown in Table II by the results of Eschenbrenner *et al.*⁶⁰

As the energy of electrons is lowered from 4 keV to 100 eV, the Born approximation gradually breaks down and impact scattering becomes dominant.⁵⁶ In other words, the excitation and ionization cross-sections increase with decreasing electron energy to reach a maximum around 60–100 eV. Such electrons have maximum LET creating a high local density (i.e., within a radius of a few nanometers) of radicals, ions, and LEE.⁶¹ This condition is statistically the most favorable to the production of two breaks on opposite strands, thus accounting for the low SSB/DSB ratio at 100 eV. However, this mechanism cannot be invoked to explain the even lower value of SSB/DSB obtained in several experiments with 10 eV electrons. At 10 eV, electron can produce very little ionization⁵⁴ so that the local density of potentially damaging species is low. However, fast electrons usually produce at least one ionization, with the outgoing electron having a high probability of lying at low energy. Thus, *a priori*, a LEE and an ion created by a high-energy photon or electron should be more prone to produce DSB than a single 10 eV electron. Then, how can we explain the SSB/DSB ratios for all energies?

Looking at the yield function for the induction of SSB and DSB with 5–100 eV electrons, provided by the work of Huels *et al.*,¹⁰ we find strong resonances in the yield of DSB at 10 and between 20 and 30 eV. Above 30 eV, multiple ionizations set in and may statistically account for low ratios. However, below 20 eV, and outside the 10 eV resonance region, the ratios of SSB/DSB are actually quite high (>15 above 12 eV and >7 below 10 eV).¹⁰ We therefore postulate that there exist energy windows (one at 10 eV and another one between 20 and 30 eV) where the electron capture and transfer mechanisms previously explained lead to a high probability of DSB formation. Within such a hypothesis, it is not sufficient for high-energy electrons or photons to produce one or many LEEs to reach a low ratio; the LEE must have the appropriate energy. We believe this explanation with the more conventional ones given in this subsection could reconcile all the data given in Table II.

V. CONCLUSION

The interaction of organic cations with DNA was found to protect the molecule from SSB and DSB induced by electrons from 1 eV to 60 keV. This interaction may decrease the capture cross-section and lifetime of transient anions formed by 1 and 10 eV electrons on bases and phosphate groups of DNA. Electron transfer from anions to Tris cations or proton transfer from Tris to anions could contribute in reducing the lifetime of transient anions formed on such DNA units. Since bond dissociation is highly sensitive to resonance parameters, such modifications could reduce DNA damage. At higher energies, a multitude of direct electron scattering processes could also be modified by the presence of organic cations within the groove of DNA. However, at 100 eV and 60 keV, the DNA molecule is less protected against SSB than at the lower energies, indicating that nonresonant mechanisms are less affected by organic salts. The reverse trend is observed for DSB induced by 100 eV electrons. At this energy, DSB may arise from the high density of LEE, radicals, and ions produced around DNA owing to the high ionization and electronic excitation cross-sections. Many of the electrons resulting from such ionizing events have low energies and hence their action is more easily quenched by organic salts. This high ionization density at 100 eV is reflected in the low ratio of SSB to DSB (~4.3) measured in present and previous experiments. It is surprising to find a similar ratio (i.e., 4.6 and 3.9 at low and high salt concentrations, respectively) for electrons of 10 eV; energy at which the ionization density is extremely low compared to that at 100 eV. This observation is explained by postulating the existence of a multistep mechanism consisting of the decay of a core-excited resonance on a phosphate group via autoionization, followed by the dissociation of the CO bond on that strand, and electron transfer to the CO bond of the opposite strand, which is ruptured by DEA.

More generally, these results indicate that transient anions formed within DNA are more sensitive to environmental changes than other mechanism of LEE-induced damage. In other words, optimum DNA damage may be reached with ionizing radiation by modifying the energy of LEE as well as the environment of DNA molecule in the cell. As recently shown by Zheng *et al.*,⁶² this sensitivity of LEE-induced damage to local environment can be exploited to understand the basic principles of radio sensitization, which may lead to better treatment of cancer with combined chemotherapy and radiotherapy.^{62,63}

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FIG. 1.

Structure and molecular weight of (a) Tris and (b) EDTA molecule, respectively.

FIG. 2.

Fluence-response curve for plasmid DNA irradiated with 100 eV electrons with salt ratio of 1:1 (■) and 6:1 (●), respectively. Each data point corresponds to the mean value of six samples $\pm$ standard deviation.

FIG. 3.

The comparison of the yields of SSB and DSB induced by 1, 10, 100, and 60 keV electrons impact on DNA films containing salt to nucleotide ratios of 1:1 (I, ●, and ▼) and 6:1 (II, ■, and ▲), respectively. The fitted curves are guides to the eye.

Yields of SSB and DSB per 10^{15} electron/molecule induced by 1, 10, and 100 eV electrons impact on five-monolayer (ML) samples of DNA (i.e., 245 ng) deposited on a tantalum substrate and by 60 KeV electron impact on 2900 nm (22.91 μg) DNA films deposited on a gold foil, respectively. Sections I and II are for the salt to nucleotide of ratios of 1:1 and 6:1, respectively. The error represents the standard deviation of six identical measurements. The percentage difference between the present results obtained with no added (I) and added salt (II) is given in the last line (ND: not detected).

TABLE I

	Form of damage	SSB				DSB				
		Energy (eV)	1	10	100	60 000	1	10	100	60 000
(I) DNA with salt of 1:1	Thickness			5 ML DNA		2900 nm		5 ML DNA		2900 nm
	This work	46±5	65±6	96±10	4.0±0.4	ND	14±4	24±3	ND	
	Reference 16 ^a		130±10							
	Reference 30 ^b		151							
(II) DNA with salt of 6:1	Thickness			9 ML DNA		5220 nm		9 ML DNA		5220 nm
	This work	27±3	39±4	69±8	3.2±0.3	ND	10±2	16±4	ND	
	Reference 16 ^a		50±10							
Difference between (I)–(II) (%)			41	40	28	20	ND	28	33	

^aYields given by Dumont *et al.* at 10 eV with the reported statistical error.

^bYields calculated from the exposure curves given by Panajotovic *et al.*

TABLE II

Comparison of the ratios of SSB to DSB obtained by different authors from irradiation of dry DNA samples with electrons and photons of different energies. When known the mole ratio of salt per nucleotide is specified. The numbers in parenthesis in the last column represent the lower and upper limits of these ratios calculated from the standard deviation of each measurement.

Reference	Source	Energy (eV)	Ratio (SSB/DSB)
This work (salt 1:1)	Electrons	10	4.6×(3.3–7.1)
This work (salt 6:1)	Electrons	10	3.9×(2.9–5.4)
Reference 10	Electrons	10	4.2×(3.3–4.6)
Reference 29	Electrons	5–20	4.3×(3.3–6.1)
Reference 34	Electrons	5–25	3–4
This work (salt 1:1)	Electrons	100	4.0×(3.2–5.0)
This work (salt 6:1)	Electrons	100	4.3×(3.0–6.4)
Reference 10	Electrons	100	3.8×(3.3–4.2)
Reference 33	Electrons	100	5.1×(3.0–9.4)
Reference 33	Electrons	500	4.8×(3.7–6.1)
Reference 33	Electrons	2000	9.6×(6.8–13.1)
Reference 33	Electrons	4000	11.7×(9.1–14.7)
Reference 58	Photons	8–25	12–20
Reference 60	X-rays	250	5.9±0.8
Reference 60	X-rays	380	5.3±0.6
Reference 60	X-rays	760	4.4±0.3
Reference 35	X-rays (calculated)	1500	10.8
Reference 29	X-rays	1500	11.4×(10.4–12.6)
Reference 36	X-rays	16000	13
Reference 36	X-ray+Auger electrons	16000	11
Reference 59	$^{60}\text{Co}(\gamma)$	~1 Mev	18