The Role of Humidity and Oxygen Level on Damage to DNA Induced by Soft X-rays and Low-Energy Electrons

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Abstract

Single- and double-strand breaks induced by soft X-rays (1.5 keV) and photo-emitted LEEs (0–30 eV) were measured in dry and humid thin films of plasmid DNA irradiated under different controllable levels of oxygen at standard ambient temperature and pressure (SATP). *G* values derived from these experiments shows that the presence of H₂O and changing the atmosphere from N₂ to O₂, while keeping all other experimental parameters constant, increases the formation of DSBs by factors of 4.5 and 11.8 for X-rays and LEEs, respectively. Under an oxygenated environment in humid DNA films, the additional LEE-induced damage resulting from the combination of water and oxygen exhibits a supper-additive effect, which leads to the formation of DSBs with a *G* value almost 7 times higher than that obtained by X-ray photons. These results indicate that O₂, H₂O and LEEs effectively contribute synergistically to enhance the formation of DSBs.

Keywords

radiation damage; single- and double- strand break; hydration level; oxygen fixation

INTRODUCTION

The interaction of high-energy ionizing radiation with a living cell results in the deposition of energy into vital biomolecules and the formation of large quantities of secondary species, such as ions, radicals, excited neutrals and non-thermal secondary electrons (SEs) within nanoscopic volumes. These highly reactive species can ultimately cause collapse of cellular functions resulting in programmed cell death (apoptosis).^{1,2} Non-thermal electrons are the most abundant of reactive secondary species produced along with the primary radiation track and carry most of the primary radiation energy. The vast majority of these electrons have energies below 30 eV with an initial energy distribution lying near 9–10 eV.^{3,4} Even when formed with energies less than that required for ionization of DNA, these low energy electrons (LEEs) induce genotoxic damage, e.g., single- and double- strand breaks (SSBs and DSBs), via resonance mechanisms, chiefly through dissociative electron attachment (DEA).⁵ In DNA, DEA is characterized by the initial capture of an electron in a vicinity of a

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subunit of DNA, to form a temporary negative ion (TNI) of that subunit, which subsequently dissociates into a radical and an anion. Since the overriding goal of radiobiological studies is to develop medical applications of ionizing radiation and the most significant effect of ionizing radiation arises from radiation-induced genomic DNA damage,^{6,7} the major mechanisms involved in the induction of damage in DNA by radiation, in particular by LEEs, have been the subject of intense investigations in recent years.⁸

Within cells, the effects of ionizing radiation on DNA are usually attributed to the sum of two indiscriminately destructive processes, referred to as direct and indirect damage. Direct damage refers to the unavoidable effects which results from radiation energy being deposited directly into the DNA components (i.e., nucleobases, 2-deoxyribose, and phosphate groups)⁹ and predominates in experiments with DNA deeply frozen (-80°C) or dried. In contrast, the indirect damage concerns the interaction of primary radiation and SEs with the local molecular environment surrounding the DNA, i.e., water, salts, proteins and oxygen molecules.^{10,11,12} The over-whelming majority of lesions in DNA irradiated in aqueous solution are caused by the indirect effect of reactive oxygen species (ROS), principally short-lived hydroxyl radicals ($\text{HO}^{\bullet}$) formed from water that diffuse to and react with DNA.¹³ However, in cells and viruses, DNA is bound and condensed by histone proteins, which is further organized into a higher-order chromatin structure. Such a configuration is surrounded by a minimal amount of water and DNA is highly protected from ROS.^{14,15} Furthermore, there is a high concentration of $\text{OH}^{\bullet}$ -scavengers and electron trapping sites near DNA. Owing to these reasons, which show the complexity of the radiation-induced DNA damage and their highly deleterious properties to cells, it is still difficult to determine experimentally,^{16,17} the relative contribution of the indirect and direct modes of damage in cells. A theoretical approach taken by Micheals et al.¹⁸ estimated a contribution value of about 45% for the direct effect and 55% for the indirect effect, when a contribution of solvated electrons to the indirect effect was not taken into account, otherwise the calculated contribution of the indirect effect would rise to 69%. C. von Sonntag et al.¹⁹ has estimated that the direct effects contribute about 40% to cellular DNA damage, while the effects of water radicals amount to about 60%. The work of Krisch et al.¹⁴ on the production of strand breaks in DNA initiated by $\text{HO}^{\bullet}$ attack sets the indirect effect contribution at 50%.

Since cellular DNA lies in a medium containing essentially water, the effects of ionizing radiation on this molecule should in principle be investigated in an aqueous environment.⁸ Studies have shown that the exposure of DNA to ionizing radiation either in frozen aqueous solutions or as dry films is not appropriate for looking at the formation of the final degradation products in terms of biological relevance. For instance, evidence provided from the identification of the diamagnetic products of nucleobases and 2-deoxyribose formed in cellular DNA indicate that aqueous solutions constitute better model systems for the indirect effects of radiation.²⁰ In contrast 5,6-dihydrothymine and 5,6-dihydrocytosine which are the main degradation products of pyrimidine bases in dry DNA samples exposed to either ionizing radiation or LEEs are not formed at least in detectable amounts in cellular DNA. This clearly indicates that dry conditions strongly modify the chemical reactions of initially generated DNA radicals.^{21,22} On the other hand, LEEs have a very short effective range of the order of 10 nm in biological media. This property of LEEs requires working with targets as thin and as uniform as possible to avoid charging by thermalized electrons. LEE

experiments are therefore usually performed with dry thin films of DNA or other biomolecules condensed or deposited on a metal substrate.^{8,23}

The gas- and condensed-phase experiments with DNA and its constituents are mostly performed under ultrahigh vacuum (UHV) conditions to prevent environmental impurities and allow one to generate LEE beams with a well-defined energy from monochromators. The results of such experiments provide information on the direct effects of LEEs. They are also required to reveal the fundamental mechanisms of action of LEEs on DNA and their role in DNA strand breaks.^{24,25,26} However, only a few advances have been made on the indirect effect of LEEs. Furthermore, the results of vacuum experiments do not necessarily correspond to those that would be observed in the complex and dynamic molecular environment of the cell. Thus, to understand how the fundamental mechanisms in radiation-DNA interactions are modified in living cells, a series of experiments have been initiated to add to DNA other vital cellular components found in the cell nucleus, such as the DNA molecule embedded into more realistic environments containing water, oxygen and DNA-binding proteins.²⁷

As the cell contains 70–80% water molecules, the majority of the radiation energy deposited in it, is absorbed in the cellular water. The yields of SSBs and DSBs in DNA by γ -rays in aqueous solution are typically 2–3 orders of magnitude greater than those for dry DNA.²⁸ Thus, in experimental and theoretical studies of DNA alteration by LEEs, recent experiments were performed with multilayer films of various form of amorphous ice in contact with DNA subunits and short oligonucleotides to simulate water molecules in an aqueous environment.^{29,30,31,32} For instance, Ptasińska and Sanche condensed 3 monolayers (ML) of amorphous ice of different isotope content (H_2^{16}O , D_2^{16}O and H_2^{18}O) onto 2 ML of the tetramer GCAT.³³ They measured the electron-stimulated desorption (ESD) yields of different anions desorbed by 3–20 eV electron impact on GCAT films under hydrated conditions corresponding to 5.25 water molecules per nucleotide. Their results confirmed that adding water to dry DNA results first in the binding of the molecule to the phosphate group, at the position of the negatively charged oxygen,³⁴ and that the formation of a DNA- H_2O complex leads to a new type of dissociative core-excited transient anion via electron capture by the complex. Theoretical studies were subsequently carried out on the diffraction of 5–30 eV electrons in hydrated B-DNA 5'-CCGGCGCCGG-3' and A-DNA 5'-CGCGAATTCGCG-3' sequences by Orlando et al.³⁵ They postulated that compound H_2O -DNA states may contribute to the damage and modify the SSB and DSB yield functions.³⁶

O_2 is another molecule present with DNA in the cell nucleus; one that is known to be an efficient radiosensitizer. Its presence in cultured mammalian cells significantly increases radiation-induced damage; since the dose of X-rays required to inactivate a given cell population to a certain level in the absence of oxygen (in a nitrogen atmosphere) can be three times higher than in its presence.^{2,37,38} Furthermore, when plasmid DNA is surrounded by oxygen at atmospheric pressure, the damage to DNA induced by LEEs is found to double.³⁹ Additionally, irradiation of the DNA films prior to the deposition of O_2 has shown that O_2 reacts with DNA sites damaged by LEEs and forms adducts with DNA radicals, which is a manifestation of the so-called oxygen fixation hypothesis (OFH), an important process in biological oxygen radio-sensitization.⁴⁰ More precisely, short-lived free radicals

(DNA^{*}) arising from the direct interaction of X-rays and LEEs in DNA, react with available O₂ molecules and generate an organic peroxide through the transient formation of a peroxy radical (DNA-OO^{*}),⁴¹ which is a non-restorable form of the DNA and more difficult for the cell to repair.^{6,38} The oxygen is said to 'fix' or make permanent the damages formed by radiation. Also, the transient species formed by indirect effects that predominantly involve ^{*}OH is giving rise efficiently to a wide variety of hydroxyl radical-mediated oxidation of the main purine and pyrimidine bases of DNA.¹³

Here, we report the results of experiments that were specifically designed to determine the contribution and efficiency of the indirect effects of LEEs on DNA at standard ambient temperature and pressure (SATP) under various relative humidity (RH) and oxygen levels. Due to the advance in our technique, we are able to isolate plasmid DNA films at SATP as dry (RH < 0.1%) and humid (RH = 100%) thin films deposited on tantalum and glass substrates and expose them to soft X-rays (effective energy of 1.5 keV) under different levels of O₂. In these experiments, for dry thin films $\Gamma \sim 2.5$, while for humidified thin films $\Gamma \sim 33$, where Γ is the hydration level given in mole of H₂O per mole of nucleotide. Comparison is made between the yields of loss of supercoiled plasmid DNA and formation of SSBs and DSBs in dry and humid DNA at different oxygen levels. Our results provide evidence that DNA strand breaks induced by LEEs are enhanced by increasing the oxygen level, particularly in the presence of bulk water molecules.

EXPERIMENTAL METHODS

Plasmid DNA samples preparation

The experiments were performed with double stranded plasmid DNA extracted from bacteria. Owing to the supercoiled configuration, a single bond rupture in a plasmid DNA of a few thousand base pairs can cause a conformational change in the geometry of the DNA, and hence can be detected efficiently by electrophoresis. A detailed description of sample preparation can be found in our previous articles.³⁹ Supercoiled DNA [pGEM-3Zf(-)] bacterial plasmid DNA, 3197 base pairs, ca. 1.97×10^6 amu, Promega was obtained from *E. coli* JM109 host and stored at -20 °C in TE buffer. It was then purified and resuspended in nanopure water having a resistivity of 18.2 M Ω .cm, without any Tris or EDTA. Its concentration was measured spectrophotometrically by measuring its absorbance at 260 nm, assuming a molar absorption coefficient of 5.3×10^7 L.mol⁻¹.cm⁻¹ at pH 7.0 for DNA, which depends on the composition and molecular weight of DNA.⁴²

Owing to the strong attenuation coefficient of soft X-rays⁴³ and the very short ranges of LEEs,³ to prevent excessive charging in targets, DNA films should be prepared as thin and uniform as possible onto substrates by lyophilization (freeze-drying). To do so, 10 μ l of plasmid solution (in nanopure water) at a concentration of 50 ng/ μ l was spread out on clean tantalum and glass surfaces and freeze dried at -70°C under the pressure of 1–3 mTorr. The frozen-dried sample had a ring shape of 6.0 ± 0.2 mm average diameter. Assuming a density of 1.71 g/cm³ for plasmid extracted from *E. coli*,⁴⁴ the mean thickness was calculated to be about 10 nm, i.e., a thickness of approximately 5 ML for a film of DNA lying parallel to the substrate.⁴⁵

Sample irradiation

We have recently developed a novel technique that permits the exposure of DNA thin films under controlled atmospheres of pure gases such as N_2 and O_2 at SATP and different levels of hydration with soft X-rays (1.5 keV) and photo-ejected secondary LEEs from a metal surface exposed to X-rays. This apparatus has been applied to similar studies and already described elsewhere in detail.^{39,46} Frozen-dried DNA films deposited on tantalum and glass substrates are transferred to this apparatus for exposure to X-rays of varying fluence. X-ray photons interacting with metal atoms produce energetic photoelectrons and Auger electrons inside the metal (mainly via the photoelectric effect). These electrons lose energy, producing at the surface a distribution consisting essentially of LEEs. The emitted SE energy distribution from a tantalum substrate, given in our previous work,³⁹ exhibits a peak at 1.4 eV and has an average energy is 5.85 eV. The average energy for SEs with energies less than 10 eV is 3.1 eV and more than 95% of photo-emitted electrons from the tantalum have energies below 30 eV. Whereas the damage yields for DNA deposited on glass are due to soft X-rays, those arising from DNA deposited on the metal are due to the interaction of both X-rays and produced LEEs emitted from the metal. The differences in the yields of strand break damage in samples deposited on the two different substrates can be attributed to the interaction of LEEs with the DNA and its surrounding atmosphere.

In this current work, dry thin films of DNA, were kept at $RH < 0.1\%$ in an irradiation chamber by filling and flushing with dry N_2 or O_2 during the irradiation. At such low humidity levels only 2.5 water molecules per nucleotide are bound to DNA. Those 2.5 water molecules are part of the structure of DNA and cannot be removed by lyophilization.⁴⁷ DNA films were humidified by exposing the freeze-dried samples to water vapor under atmospheric pressure at 100% humidity level, i.e., dried films were kept inside the chamber with circulation of N_2 and O_2 , while a water dish was placed inside the sealed chamber to maintain a specific RH condition.⁴⁸ The humidity was monitored by a traceable hygrometer sensor (Fisher Scientific) and the temperature during the irradiation is $23 \pm 1^\circ C$. For each group of films, three films were used as controls, i.e., were lyophilized, kept under the same experimental conditions as the irradiated samples, and recovered, but were not irradiated by X-rays.

Quantification of DNA damage

After exposure to radiation, samples were immediately retrieved from the chamber and dissolved in TE buffer from substrates with 95–98% efficiency. The fractions of various forms induced in DNA by irradiation was then determined by a 1% agarose gel electrophoresis run in TAE buffer (40 mM Tris acetate, 1 mM EDTA, pH 8.0) at $10 V.cm^{-1}$ for 7 min and $7.5 V.cm^{-1}$ for 68 min. The relative proportion of each various DNA configurations, quantified using ImageQuant software (Molecular Dynamics), was expressed as a percentage of the total amount. Undamaged DNA is in a compact supercoiled form (SC) that converts into a relaxed form or ‘nicked circular’ (C) form by induction of a single strand break (SSB), while induction of a double strand break (DSB) within both the SC and C forms changes the plasmid into its linear (L) form. In the present study, the purified plasmid DNA before irradiation, consists of about 96% SC form, 3% concatemeric, and 1% circular form.

Calculation of the yields of DNA damages and G values

In radiation chemistry, the damage yields can be expressed in terms of G value, i.e., the number of modified molecules or products per unit of energy absorbed by the target.⁴⁹ In the present work, the G values for 1.5 keV soft X-rays and LEEs are calculated from the slopes of exposure-response curves, which can be obtained directly from quantifying configurational changes of the DNA by measuring the percentage of the loss of SC form or formation of strand breaks as a function of photon fluence.

A freeze-dried film of pGEM-3Zf(-) plasmid DNA, with no salt and $\gamma = 2.5$, has a molecular weight of $M_W = 2.25 \times 10^6 \text{ g/mol}$ and its mass attenuation coefficient for 1.5 keV X-rays is calculated to be $\mu/\rho = 1056 \text{ cm}^2/\text{g}$, based on the atomic composition of DNA and μ/ρ of individual atoms.⁴³ For hydrated DNA films at 100% RH, the attenuation coefficient for 1.5 keV X-rays is calculated to be $\mu/\rho = 1022 \text{ cm}^2/\text{g}$. In both cases, for a 5-ML DNA film, only 0.2% of the soft X-ray photons arriving at the film's surface interact with DNA, while 99.8% pass through the film to reach the substrate. Additional photon absorption by H_2O and O_2 molecules diffused in the layer of DNA film does not change subsequently this percentage. Other parameters needed to calculate G values are $S = 0.283 \text{ cm}^2$, $t = 10 \text{ nm}$ and $\rho = 1.71 \text{ g/cm}^3$,⁴⁴ which are the area, the thickness and the density of the plasmid DNA films, respectively.³⁹

The slopes of the linear-least-squares fit of respective exposure curves represent the yields of damages (Y) induced at each film thickness. Thus, the number of damaged DNA induced in each film for a given photon fluence ($\Phi = 10^{12} \text{ photons/cm}^2$) is given by $D_{GI} = |Y_{GI}| \cdot \Phi$. N_{DNA} and $D_{Ta} = |Y_{Ta}| \cdot \Phi$. N_{DNA} , where $|Y_{GI}|$ and $|Y_{Ta}|$ are the slopes of the dose-response curves for films deposited on glass and tantalum, respectively, and N_{DNA} is the number of DNA molecules in each sample, which is calculated via

$$N_{DNA} = \frac{m \text{ (ng)} \cdot N_A \text{ (mol}^{-1}\text{)}}{M_W \text{ (g/mol)}} = 13.36 \times 10^{10} \quad (1)$$

where N_A is the Avogadro's Number and m is the mass of DNA in the sample. Hence the number of absorbed photons in the DNA film could be calculated by

$$X_{Abs} = \Phi \cdot S \cdot \left(1 - e^{-\frac{\mu}{\rho} \cdot \rho \cdot t}\right) \quad (2)$$

and the number of photons passing through the film to produce photoelectrons at the tantalum substrate by

$$X_{Trans} = \Phi \cdot S \cdot \left(e^{-\frac{\mu}{\rho} \cdot \rho \cdot t}\right) \quad (3)$$

The G value is commonly expressed in two different units, $D/100$ eV and nmol/J or $\mu\text{mol/J}$, where D represents one damaged molecule and $1 D/100 \text{ eV} = 103 \text{ nmol/J}$.⁴⁹ Thus, from our results, G value for Al K_{α} X-rays is given by

$$G_x = \frac{D_{Gl}}{1486 (eV) \cdot X_{Absorbed}} \cdot 100 (eV) \quad (4)$$

Considering $\eta_e = 0.047 \pm 0.005 \text{ e/photon}$ as the number of secondary electrons induced by photon entering the tantalum substrate,³⁹ therefore, by subtracting the damage from tantalum and glass, G value for LEEs, G_{LEE} , can be calculated by

$$G_{LEE} = \frac{(D_{Ta} - D_{Gl})}{5.85 (eV) \cdot \eta_e \cdot X_{Transmittel}} \cdot 100 (eV) \quad (5)$$

G_X and G_{LEE} are calculated within 20% and 25% errors, respectively, which mostly arise from the uncertainty on the area of the DNA film and its thickness, photon fluence, as well as the concentration of DNA in the solution and η_e .^{23,48,52}

RESULTS

In our experiments, damage to plasmid DNA is measured in the form of strand breaks or decrease of the SC plasmid population. Figure 1(a–f) presents exposure response curves for the disappearance of SC and the formation of nicked circular and linear DNA corresponding to the formation of SSB and DSB, in dry and humidified DNA samples (with 0.0 and 100% RH) deposited on glass and tantalum substrates and irradiated with X-ray photons under laboratory air atmosphere (20% O_2 + 80% N_2) at SATP. As well, Figure 2(a–c) indicates the variation in the loss of SC and the formation of circular and linear DNA as a function of photon fluence in the humid films (100% RH) under a pure O_2 atmosphere. Each point in these curves corresponds to the mean value of the yields from three samples, prepared under identical conditions; the error bars denote the standard deviation of three repeated experiments. These data are fitted with a non-linear regression (an asymptotic function). Furthermore, the formation of linear form of DNA, in all levels of oxygen exhibits a linear behavior in both dry and humid DNA films; in this case, a simple linear regression (linear-least-squares fit) is fitted to the data.

The slopes of the fitted curves at zero fluence provide the yields of loss of SC form and DNA strand breaks induction per photon fluence, for samples deposited onto glass (Y_{Gl}) and tantalum (Y_{Ta}) in various O_2 levels, as presented in Table 1. The results from our previous study with the same apparatus under pure N_2 (at 0.0% and 100% RH) as well as, under pure O_2 (at 0.0% RH) are presented in this table for comparison.^{39,48,50} The Γ value of 2.5 for the 0.0% humidity (dry DNA films) corresponds to tightly bound hydration water molecules attached to the sodium phosphate group that cannot be removed via vacuum desiccation of the DNA films, while at 100% RH, $\Gamma = 32.7$ and corresponds to the addition of an unbound

layer of molecules, which resemble bulk-like water. Our data shows that, as expected, when the X-ray fluence rises, the amount of SC decreases and inversely, the formation of SSBs and DSBs grows over the entire range of photon fluence. Additionally, the formations of circular and linear form of DNA are always greater in DNA films deposited on a tantalum than that onto a glass substrate. The damage to DNA deposited on the glass substrate is attributed to energy absorption from X-rays, whereas that produced on a metal substrate (tantalum) is induced by energy absorption from both the soft X-ray photons and secondary LEEs photoemitted from the tantalum surface. Hence these results indicate that LEEs emitted from the tantalum surface enhance DNA damage. According to our previous studies,³⁹ moreover, gaseous nitrogen does not promote radiation-induced damage to DNA, particularly strand breaks and we can assume that the direct effects of radiation in the cell correspond to the damage induced by X-rays in dry DNA under N₂. Thus, comparison of Y_{Gl} and Y_{Ta} in the absence of O₂ in dry and humid films shows that the contributions of the indirect effect due to added water are 64% and 74% on the glass and tantalum substrates, respectively, while these contributions increase to 77% and 84% for the same samples exposed under pure O₂ atmosphere.

Table 2 lists the G values for loss of SC as well as the formation of circular and linear DNA by 1.5 keV X-rays (G_X) and LEEs (G_{LEE}), in nmol.J⁻¹, derived from the yields given in Table 1. G values obtained previously with the same apparatus under pure N₂ (at 0.0% and 100% RH) as well as, under O₂ at 0.0% RH are also given in Table 2 for comparison.^{39,48,50} The G values for our 5-ML films were calculated from the yields given in Table 1 by the method already described in our previous article³⁹ and experimental setup section of this work. It should be remembered that over the spatial variation of the thickness of 5-ML films spots may exist where the local thickness is smaller than the thermalization distance of LEEs.⁵¹ For local thicknesses smaller than 5 ML (~10 nm), complete absorption of the energy of LEEs does not occur. Thus, the calculated G values in 5-ML films are underestimated, if one assumes that all the energy of the interacting electrons is absorbed in the DNA layers. Since, this problem was addressed previously, the calculated G values in the current work are corrected according to the method described in the work of Alizadeh et al.^{23,52}

Data presented in Table 2 show that G values for loss of SC induced by soft X-ray and LEEs in dry films irradiated under 100% oxygen are respectively, 1.9 and 1.8 times higher than that with no oxygen. In contrast, in humid films under an oxygenated atmosphere, G values are 813 ± 162 nmol/J and 5722 ± 1488 nmol/J for X-rays and LEEs, respectively, i.e., the G values are 3 and 3.1 times higher than the corresponding value in a nitrogenated environment, for X-ray and LEEs, respectively. Since in our experiments the loss of supercoiled DNA in both dry and humid films and onto different substrates happens mostly via formation of circular DNA, the same behavior is observed in G values for formation of SSB. However, more surprising results relevant to the DSB formation presented in Table 2, when in humid DNA films the G values are 36 ± 7 nmol/J and 2472 ± 64 nmol/J for X-rays and LEEs, respectively, i.e., 4.5 and 11.8 times higher than the corresponding values for X-ray and LEEs, respectively, for the same films in the absence of oxygen. For a clearer comparison of X-ray photons and LEE effectiveness in inducing of SSBs and DSBs in dry and humid films, we define an enhancement factor (EF) as the ratio of G_{LEE} to G_X . Figure 3

presents the dependence of EF to the level of oxygen for loss of SC and formation of SSB and DSB damage in dry and humid DNA films. It clearly shows that within the experimental errors, by increasing the O_2 level from 0.0 to 100%, EF s for loss of SC and SSB formation have almost a constant average around 6–7 for humid films and around 2 for dry films. The EF s for the formation of DSBs strongly depend on the presence of O_2 and substantially increase in humid films irradiated under 100% oxygen, indicating that the combined effect of water molecules and oxygen is synergistic in causing DNA damage.

DISCUSSION

After considerable work by Cai et al.,⁵³ Brun et al.⁴⁶ and Alizadeh et al.³⁹ it has been established that LEEs have much higher G values than X-rays for damaging DNA. From these studies, it was concluded that SEs emitted from tantalum with an energy distribution peaking around 1.4 eV can cause considerable DNA damage via DEA, resulting in larger yields of damage for DNA films deposited on tantalum than that on glass substrate. Mechanisms to explain the higher yields of damage induced by LEEs than by X-ray photons in vacuum-dried films have been extensively discussed in previous articles.^{39,46} Furthermore, experiments at different humidity levels confirmed that DNA damage increases with the level of hydration. A dramatic increase in LEE-induced damage as hydration levels approach of bulk-like hydration conditions, was observed indicating a considerable contribution to *indirect* DNA damage.⁴⁸ This contribution does not substantially arise from water molecules in contact with DNA, but mostly from highly reactive species created within the subsequent bulk-like water layer or at the interface between the latter layer and the water of hydration. We found that in the vicinity of DNA, when a SE reaches sufficiently low energy to enter an interface trap, it may be scavenged in regions of large electron affinities such as those around nucleobases. As shown experimentally⁵⁴ and theoretically,⁵⁵ TNIs of the DNA bases have a strong tendency to transfer the extra electron to the phosphate group, where it resides in the usually unfilled σ^* orbital of the C-O bond. The potential energy surface of that state being dissociative, the C-O bond ruptures causing a SSB. This process could therefore contribute to the high yields of SSB found at 100% RH. Thus, we proposed that this huge enhancement in DNA damage with addition of bulk like water may be essentially due to two mechanisms: 1) a rise in the amount of the species H^- , $OH^\bullet$ and $H^\bullet$ in bulk water and 2) the slowing-down of SEs to energies close to that of the conduction band edge of amorphous water, followed by their transient capture into shallow traps at the interface between the hydration water layers of DNA and bulk water. Owing to modification of the adiabatic electron affinities of the DNA bases by surrounding water molecules,⁵⁶ electrons in these shallow traps would rapidly transfer to the bases of solvated DNA to form transient anions. These anions may dissociate or transfer the excess electron to the DNA backbone to cause a strand break.⁴⁸

Since the effect on damage yields of adding water and oxygen separately to DNA have been previously investigated and discussed in our former articles,^{39,48} as well as summarized above and in the introduction of the present work, the remaining discussion is focused essentially on the effect of oxygen in the induction of damage to DNA in humid films. As presented in Tables 1 and 2, the absolute amounts of yields of damage induced by X-rays and LEEs, as well as G_X and G_{LEE} increase in dry and humid films with rising levels of

oxygen; a strong rise in DSBs occurs at 100% RH under an oxygenated environment with LEEs. More remarkable results from EF values in Figure 3 indicate that in both dry and humid films, the presence of oxygen does not change the effectiveness of LEEs with respect to soft X-rays in the induction of SSBs or loss of SC; The EF s for DSB formation behave differently, indicating that in presence of water molecules, LEEs are more effective in the induction of DSB with increasing levels of O_2 . At 100% oxygen level, the additional LEE damage resulting from the combination of water and oxygen is super-additive leading to the formation of DSBs with a G value almost 7 times higher than that obtained by X-ray photons in DNA films irradiated under the same experimental conditions. This enhancement in damage must therefore involve the almost simultaneous action of O_2 , H_2O and LEE on DNA, as tentatively explained in the next paragraph.

In the present experiments, the number of induced DSBs is found to be linear with dose. This finding implies that the observed simultaneous presence of two SSBs in opposite DNA strands within one helical turn (i.e., a DSB)⁵⁷ is the result of a single triggering event. It is well-known that between two types of radiation-induced DNA damages, i.e., base damage (or loss) and sugar damage, the former one does not appreciably alter the structure of the DNA molecule, while sugar and phosphate lesions, frequently disrupt promptly the sugar-phosphate backbone and generate SSBs.¹⁹ Since DEA is the main process involved in the LEE-induced breakage of chemical bonds between DNA basic constituents,⁵⁸ it seems logical to propose that DEA may be involved in the vicinity of O_2 and H_2O in the formation of DSBs in DNA films containing these molecules. $H^\bullet$ could be initially produced in large quantities from nucleobases or the 2-deoxyribose undergoing DEA at sub-excitation energies (< 3 eV).^{59,60,61} The electron remaining on the base or sugar unit could then transfer to the CO bond of the phosphate group, as it is usually the case,⁵ and break one strand. The other adjacent strand could possibly be broken via the interaction of the liberated hydrogen atom with O_2 , which in our case could be formed by the reaction $H^\bullet + O_2 \rightarrow HO_2^\bullet$; Although $HO_2^\bullet$ and related $O_2^{\bullet-}$, which is predominant in aqueous solution and exhibits a low reactivity with most molecules at the exception of thiols.^{62,63} The only reactions of $O_2^{\bullet-}$ with DNA involved competitive reduction and addition to the highly oxidizing radical that arises from the deprotonation of the guanine radical action. More $O_2^{\bullet-}$ has been also observed with the oxidizing radical arising from one-electron oxidation of tyrosine.⁶⁴ Additionally, it is well established that solvated electrons that are produced by either radiolysis of water molecules or ionization of biomolecules in aqueous environment are effectively scavenged by O_2 at a diffusion controlled rate giving rise to the unreactive superoxide radical $O_2^{\bullet-}$.⁶⁵ Under aerobic conditions, organic radical anions formed by the addition of non-scavenged electrons to nucleobases are efficiently oxidized by O_2 leading to the regeneration of the starting biomolecule together with the formation of $O_2^{\bullet-}$.

Finally, we point out that the metal substrate may also be partially involved in O_2 sensitization with H_2O , since in our 5-ML films, about 20% of the DNA is in contact with tantalum. Oxyl radicals derived from reactions with the metal substrate and H_2O_2 formed in the water⁶⁶ should be considered for the DNA layer in contact with the tantalum under oxygenated atmosphere. It has been shown that H_2O_2 acts as a much stronger oxidant when decomposed by some metal ions, such as ferrous, nickel (II) and cobalt (II), resulting in

DNA damage via the Fenton reaction. The H_2O_2 does not react directly with DNA, but results in the formation of $HO^\bullet$ in the presence of certain metal ions^{67,68} especially those of iron and copper.⁶⁹ Studies have shown that metals may generate reactive oxygen species from hydroperoxides which arise on DNA sugars and bases and may oxidize these components.⁷⁰

SUMMARY

We have investigated the DNA damage induced by soft X-rays (1.5 keV) and photo-emitted LEEs (0–30 eV) in dry and humid thin films of plasmid DNA irradiated under different controllable level of oxygen at SATP. G values were derived from these experiments for DNA strand breaks induced by X-rays and LEEs under conditions closer to those of the cell than possible in previous experiments. Our data shows that G values for loss of SC (or formation of SSB) induced by X-rays and LEEs in dry films irradiated under 100% oxygen are respectively, 1.9 and 1.8 times higher than that with no oxygen. In humid films under an oxygenated atmosphere, the G values for loss of SC are 3 and 3.1 times higher than the corresponding value in a nitrogenated environment, for X-rays and LEEs, respectively. The formation of DSBs happens at different rates with respect to SSB formation. In the presence of H_2O , changing the atmosphere from N_2 to O_2 , while keeping all other experimental parameters constant, increases the formation of DSBs by factors of 4.5 and 11.8 for X-rays and LEEs, respectively. These findings not only provide an insight into the radiosensitization mechanism of O_2 , but also reveal the significant role of LEEs to enhance efficiently the formation of DSBs. This type of damage splinters the genome which can result in genomic alterations causing mutations, chromosomal aberrations or cell death.

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Figure 1.

Exposure-response curves for DNA irradiated by 1.5 keV X-rays, under air atmosphere (80% N₂ + 20% O₂) and two different hydration levels (RH %) at SATP for: (a–b) the loss of supercoiled DNA; (c–d) formation of circular DNA (SSB), and (e–f) formation of linear DNA (DSB). The data are shown for 5-ML films of plasmid DNA deposited on glass (▼) and tantalum (■). Each point corresponds to the mean value of the yields from three samples, prepared under identical conditions; the error bars denote the standard deviation of the three experiments. The solid lines exhibit the linear behavior at low fluence and the dash lines exhibit the fit to an exponential function.

Figure 2.

Exposure-response curves for DNA irradiated at SATP by 1.5 keV X-rays under 100% O₂ at 100% RH. From top to bottom: loss of supercoiled DNA and formation of circular (SSB) and linear DNA (DSB), respectively. The data are shown for 5-ML films of plasmid DNA deposited on glass (▼) and tantalum (■). Each point corresponds to the mean value of the yields from three samples, prepared under identical conditions; the error bars denote the standard deviation of the three experiments. The solid lines exhibit the linear behavior at low fluence and the dash lines exhibit the fit to an exponential function.

Figure 3.

Dependence of enhancement factor (EF) to the level of oxygen for the loss of supercoiled DNA and formation of circular (SSB) and linear DNA (DSB) in dry and humid DNA films. EF is defined as the ratio of G_{LEE} to G_X .

Table 1
Percentage Yield Per 10^{12} photons. cm^{-2} for the Loss of SC and Formation of SSBs and DSBs in Dry and Humid DNA Films Deposited on Tantalum and Glass Substrates Irradiated by 1.5 keV X-rays under Different Levels of O_2 at SATP

$\frac{\text{RH}(\%) \rightarrow}{\downarrow \text{O}_2(\%)}$		0.0% ($\Gamma = 2.5$)			100% ($\Gamma = \sim 33$)		
	DNA Form	Y_{Gl}	Y_{Ta}		Y_{Gl}	Y_{Ta}	
0.0% (N_2)	SC	3.6 ± 0.6	4.9 ± 0.7		10 ± 1	19 ± 5	
	SSB	3.3 ± 0.3	4.5 ± 0.4		9.0 ± 1	18 ± 5	
	DSB	Not detected	0.3 ± 0.1		0.3 ± 0.02	0.4 ± 0.04	
20% ($\text{N}_2 + \text{O}_2$)	SC	5.1 ± 0.5	6.5 ± 0.7		12 ± 1	21 ± 3	
	SSB	5.0 ± 0.3	6.0 ± 0.3		11 ± 1	20 ± 3	
	DSB	0.3 ± 0.1	0.35 ± 0.1		0.3 ± 0.1	0.45 ± 0.1	
100% (O_2)	SC	6.8 ± 0.9	9.1 ± 0.8		29.7 ± 5	57.5 ± 11.7	
	SSB	6.4 ± 0.7	8.5 ± 0.5		27.4 ± 4.9	54.5 ± 10.7	
	DSB	0.3 ± 0.1	0.4 ± 0.1		1.3 ± 0.3	2.5 ± 0.5	

The *G*-values for the Loss of SC and Induction of SSB and DSB by Soft X-rays and LEEs in Dry and Humid DNA Films Irradiated by 1.5 keV X-rays under Different Levels of O₂ at SATP

Table 2

$\text{RH}(\%) \rightarrow$ $\downarrow \text{O}_2(\%)$		0.0% ($\Gamma = 2.5$)			100% ($\Gamma = \sim 33$)		
		DNA Form	$G_X(\text{nmol/J})$	$G_{LEE}(\text{nmol/J})$	$G_X(\text{nmol/J})$	$G_{LEE}(\text{nmol/J})$	
RH 0.0% (N ₂)	SC		98 ± 20	260 ± 50	274 ± 55	1852 ± 482	
	SSB		93 ± 19	248 ± 65	246 ± 49	1852 ± 482	
	DSB		-	8 ± 2	8 ± 2	21 ± 5	
RH 20% (N ₂ + O ₂)	SC		140 ± 28	288 ± 75	328 ± 66	1852 ± 482	
	SSB		137 ± 27	206 ± 54	301 ± 60	1852 ± 482	
	DSB		8 ± 2	10 ± 3	8 ± 2	31 ± 8	
RH 100% (O ₂)	SC		186 ± 37	473 ± 123	813 ± 162	5722 ± 1488	
	SSB		175 ± 35	432 ± 112	750 ± 150	5577 ± 1450	
	DSB		8 ± 2	21 ± 5	36 ± 7	247 ± 64	