Measurements of *G*-values for DNA Damage Induced by Low Energy Electrons

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Abstract

We address the problem of measurement of *G*-values (damage per deposited energy) for low energy electrons (LEEs) below 30 eV. Such values (G_{LEE}) usually have to be derived from damage yields in nanometer (~10nm) thick films, which are too thin to allow complete absorption of the energy of LEEs. In this work, we determine optimum corrections to obtain reliable G_{LEE} in 2–80 nm thick films of plasmid DNA which are not uniform. G_{LEE} is found to increase with film average thickness and reaches a plateau at 260 ± 50 nmol/J around 20 nm, which corresponds to the most reliable value. The previously G_{LEE} measured for films thinner than 20nm that were underestimated can be corrected from a factor derived from the present results. This method could be used to obtain reliable G_{LEE} for other biomolecules so as to be able to compare LEE-induced damage to that produced by other types of radiation under various experimental conditions.

Keywords

Radiation damage; *G*-values; Low energy electrons; DNA; Strand breaks

1. Introduction

Cell killing by ionizing radiation is associated with structural damage to cellular DNA via the formation of lesions such as strand breaks, base modification and cross links.^{1–3} For this reason, radiation physics and chemistry studies have attempted to link biological damage to fundamental processes including the production of electrons, ions, electronic excited states^{4–6} and subsequent reactions.^{1,6–11} Many studies involved the reactions of radiation products in aqueous solutions with the DNA molecules.^{1,6–9} To compare the yields of damage in the different experimental models radiation chemists measure a universal quantity referred to as the *G*-value. Thus, the damage imparted by high energy radiation is often quantified by measuring the *G*-value, which corresponds to the number of moles of substance produced per joule of radiation energy absorbed.¹² The *G*-value depends not only on the target, but also on the energy and type of radiation. Most of these *G*-value measurements have been performed with high energy radiation under different experimental conditions. For example, the *G*-values for single strand breaks (SSBs) induced in DNA have

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been measured at various energies for X- and γ -rays,^{12,13} α -particles,^{14,15} and high-energy electrons.¹⁶

With high energy particles or photons measurements of G -values usually do not pose any particular problem because macroscopic or at least sufficiently thick target are irradiated. With such targets, the amount of energy deposited can simply be obtained from the attenuation coefficient of the absorbing matter, since in this case the number of attenuated particles by the target, multiplied by the energy of the particles is equal to the energy absorbed. However, for nanoscale-thin targets this condition is usually not met; i.e., not all the energy of the scattered photons or particles is deposited in the sample so that the measured G -values are underestimated. In present work, we address this problem in the case of G -value measurements for DNA damage induced by low energy electrons (LEEs).

DNA damage induced by LEEs has been extensively investigated in the past decade to understand the basic mechanisms affecting the induction of biological lesions.^{3,17,18} Such electrons with initial kinetic energies below 30 eV are produced in large quantities¹⁹ and play a central role in propagating the effects of ionizing radiation.^{6,17} LEEs have thermalization distances of the order of 10 nm in biological materials, which define the initial volume of energy deposition.^{4,20} This range is comparable to the diameter of DNA (2–5 nm) and the nucleosome (10 nm).⁵ 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 films of DNA or other biomolecules condensed or deposited on a metal substrate.^{3,21} The film thickness is usually smaller than the thermalization distance of LEEs. Up to date, a fairly large number of experiments have measured the damage induced to DNA by LEEs.³ In the case of plasmid DNA,^{17,22–34} which represents the most biologically significant target, most measurements report the decrease of the intact supercoiled population or the production of single and double strand breaks for thin films of about 10 nm thickness, as a function of electron energy, under different conditions. Only in a few of these,^{32–34} have attempts been made to measure the G -values. But even in these measurements, there is no guaranty that the energy of the electrons has been completely deposited in the film and the G -values may have been underestimated. Hence there exists a need to develop a method to alleviate this problem so as to derive the best possible G -values for LEE-induced damage (G_{LEE}); particularly, if we want to compare reliably the efficiency of LEEs to damage biomolecules with other particles and photons under various experimental conditions.

Films can be bombarded with two different sources of LEEs: either a direct monochromatic electron beam impinging from vacuum onto the film surface or photoelectrons emitted from the metal substrate on which the film is deposited. The former source has the advantage of being monoenergetic, whereas the photoelectron source makes it possible to induce LEE damage to biomolecules under gaseous atmospheres at atmospheric pressure (i.e., under conditions closer to those of living cells). Studies of the damage to DNA resulting from the emission of low energy photoelectrons were introduced by Cai et al. in 2005.³² The photoelectrons (mean energy of 5.85 eV) were produced by a tantalum surface exposed to Al K_{α} X-rays of 1.5 keV, under vacuum. The apparatus and the technique were further

developed by Brun et al.³³ and Alizadeh et al.³⁴ to perform experiments at atmospheric temperature and pressure.

In the present article, we report the results of a study on the dependence of LEE induced DNA damage on average film thickness. We find that with the apparatus recently developed by Alizadeh et al.,³⁴ it is possible to explore a much larger range film thicknesses than in previous experiments. By plotting damage and the corresponding G_{LEE} as a function of thickness, we show that more reliable estimates can be obtained as well as a factor for correcting the values obtained at too small thicknesses. A compilation of corrected G -values provides solid evidence of the greater efficiency of LEEs to induce DNA damage compared to other types of radiation.

2. Experimental Methods

In the present experiments plasmid DNA films were deposited on two different types of substrate: an insulator substrate (glass), and an electron-emitting tantalum substrate. They were prepared by lyophilisation with a technique previously described in detail.³⁴ The films were irradiated with Al K_{α} X-rays of 1.5 keV. Damage produced on the glass substrate was attributed to energy absorption from X-rays,^{32,34} whereas that produced on a metal substrate arose from energy absorption from both the soft X-ray beam and secondary electrons (SEs) emitted from the tantalum surface.³⁴ From comparison of the results obtained with DNA films deposited on glass and tantalum, the damage created by LEEs emitted from tantalum is deduced and the G -values calculated for LEEs under dry N_2 at atmospheric pressure and temperature. Atmospheric pressure experiments were chosen so as to obtain G -values under conditions closer to those of radiation chemistry measurements.

2.1. Plasmid DNA Films Preparation

Supercoiled DNA [pGEM-3Zf(-) bacterial plasmid DNA, 3197 base pairs, ca. 1.97×10^6 amu, Promega] was obtained from *Escherichia coli* JM109 host, and purified using Qiagen kits. To protect the plasmid DNA from degradation, the DNA pellet was then redissolved in TE buffer (10 mM Tris, 1 mM EDTA) at pH 8. Prior to use, DNA was cleaned of TE by passing the solution through a home-made microcolumn of Sephadex G-50 resin on a bed of glass beads, to remove the small molecules of salts from the solution.^{35,36} The concentration of DNA in the filtered solution was then measured spectrophotometrically from its absorbance at 260 nm, assuming a molar absorption coefficient of $5.3 \times 10^7 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ at pH 7.0 for DNA.³⁷ DNA solutions were then diluted in ddH₂O to obtain the different concentrations of DNA required to make DNA films of variable thicknesses.

A specific volume (V) of a known concentration of plasmid dissolved in nanopure water was spread out on clean tantalum and glass surfaces and freeze dried (lyophilized) at -70°C under the pressure of 1–3 mTorr for about two hours. The dried sample had a ring shape of average radius r . Assuming a density of $\rho = 1.71 \text{ g/cm}^3$ for the plasmid extracted from *E. coli*,³⁸ the average thicknesses (t) of different groups of DNA films were determined by applying the formula

$$m_{DNA} = \rho \times V = \rho \times S \times t = \rho \times (\pi \cdot r^2) \times t \quad (1)$$

Here, m_{DNA} is the mass of DNA in each sample, and S the area of the sample. Thus, by measuring S , it was possible to deduce t , the average thickness of the sample, within a 30% error; the latter arose principally from the uncertainty on S . The spatial variations of the thickness can be estimated from previous measurements.³

Charge accumulation in films prepared by the present technique has been previously measured by monitoring the energy of the onset of the transmission of electrons through the film, from vacuum to the tantalum substrate.³ Because a positive energy shift of this onset is caused by electrons trapped in the film, it has been repeatedly possible to estimate that beyond an average thickness of ~10 nm, the samples started to charge when impinged by 1–10 eV electrons. For uniform DNA films this measurements would translate into an electron range of the order of 10 nm. Highly non-uniform samples would charge at a much lower average thickness, since 10-nm regions would be reached with less DNA. The thermalization distance of 10 eV-electrons in DNA is 12 ± 1 nm³⁹ and 11 ± 3 nm on average for 1–10 eV electrons in water ice.⁴⁰ Thus, with the present source composed mostly of electrons between 0–10 eV (see Section 2.4), we expect electrons to be trapped within a distance of 11 ± 3 nm which corresponds to the onset thickness of charging. Thus, we conclude that the spatial thickness variations in our films can not be much larger than the errors in the electrons thermalization distance (± 3 nm) and the error in the average thickness measurements (± 3 nm). Thus, according to this estimate we can expect the largest spatial variation to be 60% of the average thickness of our films.

2.2. Experimental Setup and Irradiation Conditions

The 1.5 keV Al K_{α} X-rays are generated from a cold-cathode source constructed by Alizadeh et al.,⁴¹ according to the original design of Hoshi et al.⁴² A plasma discharge with 5.5 mA current is formed between a cathode and an aluminum foil target in a vacuum chamber. Aluminum characteristic K_{α} X-rays, produced by electron bombardment of the target, travel outside the chamber through a flight tube continuously flushed with helium gas at atmospheric pressure. The X-rays then pass through a thin foil of mylar to enter a small chamber filled by dry N_2 at atmospheric pressure, where plasmid DNA films are deposited on the different substrates.

The frozen-dried films are transferred to the X-ray apparatus for exposure to X-rays of varying fluence, in the presence of nitrogen gas having a stated purity of 99.9% and no humidity as monitored by a hygrometer sensor placed in the irradiation chamber. For each group of samples, two samples are used as controls; i.e., these samples are lyophilized, kept under the same atmospheric experimental conditions as the irradiated samples, and recovered, but not irradiated by X-rays.

2.3. Quantification of the Yields by Agarose Gel Electrophoresis

After irradiation, the samples were immediately retrieved from the chamber and the tantalum and glass surfaces with 95–98% efficiency by dissolving in TE buffer. 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 configuration was expressed as a percentage of the total amount. The undamaged plasmid exists in a supercoiled topological form (SC). Induction of a SSB converts the plasmid into a relaxed form or 'nicked circular' (C) form, 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, before irradiation, 96% of the extracted plasmid was in the supercoiled form and the rest was in the relaxed circular (C, > 3%) and concatemeric (CM, < 1%) configurations.

About 100 ng of DNA from each recovered solution were prestained by 3 µL of 100× SYBR® Green I (Molecular Probes™) for loading in each well. The samples were incubated with SYBR Green I for at least 15 minutes prior to electrophoresis. The gel itself was stained by 8 µL of 10,000× concentration SYBR Green I. After electrophoresis, gels were scanned with the Typhoon-Trio laser scanner (GE Healthcare) using the blue fluorescence mode at an excitation wavelength of 488 nm and filter type 520 BP 40. Various DNA forms were quantified using ImageQuant software (Molecular Dynamics). These values were corrected using a normalization factor, because of the weaker binding of SYBR Green I to the supercoiled form of DNA compared to nicked circular and linear configurations. For pGEM-3Zf(–) plasmids used in this work, correction factor of 1.4 was determined after quantification by ImageQuant.

The number of X-ray photons incident on the samples was determined within an accuracy of 8% using GAFCHROMIC® HD-810 dosimeter films. A detailed description of the calculations and the calibration of the sensitivity of the films can be found in Ref. 34.

2.4. SE Emission from Tantalum

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 of the tantalum substrate a distribution consisting essentially of LEEs. The SE energy distribution curve from a tantalum substrate has been given in our previous work.³⁴ The electron energy distribution peaks at 1.4 eV and 95% of the electrons have energies below 30 eV. The average energy is 5.85 eV. A total electron yield of 0.049 electrons per photon is obtained from this distribution.³⁴ The current of the X-ray-induced SE emitted from a tantalum surface was measured to be 0.17 ± 0.02 nA. Consequently, the electron flux and the quantum yield of electron emission were calculated to be $(0.54 \pm 0.2) \times 10^9$ electrons.s⁻¹.cm⁻² and 0.047 ± 0.005 electron per photon, respectively. The latter was used in *G*-value calculations as η_e , the number of SEs induced per photon entering the tantalum substrate.

2.5. Calculation of G-values

The molecular weight of plasmid DNA and its mass attenuation coefficient for 1.5 keV X-rays were calculated to be $M_W = 2.25 \times 10^6 \text{ g/mol}$ and $\mu/\rho = 1056 \text{ cm}^2/\text{g}$, respectively, based on the DNA's composition and μ/ρ of individual atoms.⁴³ For each thickness, the dose-response curves (i.e., percentage loss of SC DNA as a function of incident photon fluence) for both glass and tantalum substrates were plotted from zero fluence to $25 \times 10^{11} \text{ photons/cm}^2$. Each of these dose responses curves required measurements on 3 samples at each dose for six different doses for a total of almost 360 analyses of DNA damage by agarose gel electrophoresis. The slopes of the linear-least-squares fits of respective exposure curves represent the yields of damages induced at each film thickness. Thus, the number of damaged DNA induced in each film for a given photon fluence (Φ) is given by $D_{Gl} = |SC_{Gl}| \times \Phi \times N_{DNA}$ and $D_{Ta} = |SC_{Ta}| \times \Phi \times N_{DNA}$, where N_{DNA} is the number of DNA molecules in each sample, and $|SC_{Gl}|$ and $|SC_{Ta}|$ are the slopes of the dose-response curves for films deposited on glass and tantalum, respectively. 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

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

In radiation chemistry, the G -value is commonly expressed in two different units, $D / 100 \text{ eV}$ and nmol/J or $\mu\text{mol/J}$, where D represents one damaged molecule and $1 D / 100 \text{ eV} = 103 \text{ nmol / J}$. Thus, from our results, G_{LEE} is given by

$$G_{LEE} = \frac{(D_{Ta} - D_{Gl})}{\eta_e \times X_{Trans} \times 5.85 \text{ eV}} \times 100 \text{ eV} \quad (4)$$

Eq. 4 gives G -values in $D / 100 \text{ eV}$.

3. Results and Discussion

For the thinnest DNA films in our experiments ($\sim 2 \text{ nm}$), only $> 0.1\%$ of the incident X-ray photon flux interacts directly with DNA molecules, while 99.9% passed through the DNA film to the tantalum substrate where they induce SEs from the metal surface. For the thickest film in this work ($\sim 200 \text{ nm}$), we estimate that almost 4% of the incident photons are absorbed within the DNA (i.e., 96% reach the substrate). Figure 1 shows the dependence of the number of absorbed photons versus the thickness of DNA films for an incident fluence

of 10^{12} photons/cm², calculated from Eq. (2). The error bars reflect the deviations in the measurements of the photon flux and the thickness of three different films or area of the films. X-ray absorption obeys the Beer-Lambert law and should have an exponential behaviour. However, within experimental errors, the absorption of the X-rays in DNA films can be considered to increase linearly with thicknesses below 250 nm. Consequently the number of damaged DNA molecules with film thickness is fitted to a linear function.

Figure 2 (a–b) represents the variation in the number of damaged molecules as a function of DNA film thickness. As expected, for the same thickness, the number of damaged DNA molecules for plasmid deposited on the tantalum substrates (i.e., damage induced by both soft X-rays and LEEs) is greater than that to DNA deposited on the glass (i.e., damage arising from soft X-rays). The points recorded above 200 nm in Figure 2a, which do not obey this linearity, show the limit of the instrument. Beyond 150 nm the film cannot be grown to a size that fits completely under the area of the X-ray beam.

As previously explained, DNA films deposited on tantalum were irradiated to measure the additional damage induced by photo-emitted LEEs. Since the latter have a very short effective range,⁴ there was no reason to continue measurements beyond 80 nm (Fig. 2b). This is clearly shown in Figure 3. The curve of Fig. 3 illustrates the contribution to DNA damage induced by X-rays and LEEs at different film thicknesses via an enhancement factor (EF). The EFs were derived by dividing the yield of damaged DNA on the tantalum substrate by that measured in DNA films on the glass, under the same experimental conditions. As presented in Fig. 3, for the thinnest film in our experiments, this factor is almost 1.6 and agrees well with the results of Cai et al.,³² i.e., 1.5 in monolayer films. Thus, in thinner films, the photo-emitted LEEs increase significantly DNA damage considering that only 47 ± 5 photoelectrons escape the surface per thousand incident photons. By increasing the thickness, the EFs get closer to unity, indicating a lesser relative contribution of LEEs to the damage, owing to the increased absorption of X-ray photons by an increasing the amount of DNA molecules.

Calculated G -values for loss of SC by LEEs (G_{LEE}) under N₂ atmosphere are shown as a function of thickness of DNA films in Figure 4. The solid curve is a saturating exponential fitted to the data. G_{LEE} rise rapidly as a function of thickness until they saturate at 260 ± 50 nmol/J around 20 ± 6 nm. As expected, this behaviour indicates that below 20 nm a fraction of SEs escape from the DNA film into vacuum without depositing all of their energy. For $t > 20$ nm they all seem to remain in the film and deposit their energy. In other words, sufficient material covers the tantalum substrate to absorb all electrons. As discussed in Section 2.1, the thermalization distance of most photoelectrons emanating from the tantalum surface is estimated to be 11 ± 3 nm. Thus, a film of 20 nm average thickness has spatial variations in thickness from 11 ± 3 nm to an upper bound, which can not be deduced from the data. Assuming that spatial variations are uniformly distributed around the mean thickness the local thickness varies from 11 to 29 nm along the plane of the tantalum substrate. These fluctuations correspond to a maximum variation of 45% of the mean thickness. They are smaller than those predicted from charge measurements in Section 2.1 (i.e., 60%), but if we include the error on the thermalization distance of the electrons, they become similar. These fluctuations are large, but fortunately, at and beyond 20 nm thickness, the G -values become

independent of non-uniformity, since electrons deposit all of their energy in the film. Consequently, as the film thickness increases the G -value remains constant. Thus, the present method allows deducing G -values, which are independent of spatial film irregularities. This is a considerable asset considering the difficulties in producing uniform DNA films.

We note, however, some electrons are expected to scatter back into the metal substrate without losing all of their energy in the film for thick, as well as thin films. So even through the saturation of the curve in Fig. 4 provides the most valid G -value, it still underestimates the real G_{LEE} . Undoubtedly, at thicknesses of 10 to 80 nm the film must be charging from electrons that thermalize, since in DNA the effective range of LEEs is about 11 nm.²³ However, within our experimental error, it seems that charging, which should increase considerably from 10 to 80 nm, does not modify the smooth exponentially saturating behaviour of the curve of Fig. 4. This observation and the proceeding discussion on film uniformity lend credibility to the present experiment as a reliable method to obtain the best possible G -values for LEEs.

Knowing the G_{LEE} in the plateau point allows calculating a factor to find the difference between the most reliable G -value and the one measured at too small thicknesses. This factor is calculated by

$$f = \frac{G_P - G_t}{G_P} \quad (5)$$

where f is the fraction of actual damaged induced in the films of thickness (t) which should have been caused by escaping electrons, and G_P and G_t are the G -values at the plateau and measured at t , respectively. We list in Table 1, G_{LEE} obtained under different conditions, previously recorded with the same apparatus,^{32–34,44} along with the corrected G -values

deduced from Fig. 4 or from Eq. 4, i.e., $G_P = \frac{G_t}{1-f}$. We can also consider, $CF = \frac{1}{1-f}$ as a correction factor for finding G_P for LEEs under different environmental conditions for each specific thickness. For instance, for films of 10 and 2 nm average thicknesses, CFs are calculated to be 5.9 ± 0.7 and 1.15 ± 0.03 , respectively, which correspond to 83% and 13% corrections in G_{LEE} for 2 and 10 nm films, respectively. It indicates that for thinner films the calculated G -value is much lower than G_P obtained in this work (i.e., more LEEs escape from thinner films).

Owing to the universality of G -value, we are now in a position to compare the efficiency of LEEs to damage biomolecules with that of other type of radiation under various environmental conditions. Such G -values are presented in Table 2, for the formation of SSB. As already reported in many studies and listed in Table 2, X- and γ -rays of varying energies induce damage to DNA under dilute, aqueous solutions, hydrated and dry conditions. Most of the results in this table are in good agreement with each other, such as those of Purkayastha et al.,⁴⁵ Yokoya et al.⁴⁶ and Cai et al.^{32,47} for dry DNA.

Since the formation of SSB is the main portion of damage induced by LEEs in the data of Table 1, G_{LEE} for loss of SC can be compared to G -values for SSB damage mentioned in Table 2. Thus, two tables provide the most reliable comparison of G_{LEE} for DNA with those for other type of radiations. It shows that G -values for LEEs are higher than those for photons and even high-LET radiation such as α -particles under different conditions. LEEs are found to have higher effectiveness in causing damage to DNA relative to higher energy radiation. The reasons for this higher effectiveness have been discussed in previous publications.^{32–34}

4. Conclusions

G -values for the loss of supercoiled DNA induced by LEEs with an energy distribution peaking around 1.4 eV were determined in DNA films of thicknesses ranging from ~ 2 to 80 nm. We found that the number of damaged DNA and the G -values for LEEs increase with average sample thickness until saturation is observed around 20 nm. Although LEEs have an effective range of the order of 10 nm, we found that with thicknesses < 20 nm, some of them escape into the surrounding atmosphere without depositing all of their energy in the DNA film. Thus, the calculated G -values in such thin films are underestimated, if we assume that all the energy of the interacting electrons is absorbed in the DNA layers. In this work, we presented a method to obtain more reliable G -values for LEE-induced damage to DNA that are independent of film thickness and its spatial variation. Thus, the method allows generating reliable G_{LEE} even if the spatial distribution of DNA in the irradiated sample is highly irregular. It also allows to correct previous determinations of G_{LEE} measured in too thin films. From those corrected values, the first comparison of G_{LEE} obtained under various experimental conditions could be made with those for DNA damage induced by other types of radiation.

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Figure 1.
Dependence of the number of absorbed X-ray photons as a function of thickness of DNA films for incident fluence of 10^{12} photons/cm².

Figure 2. Number of damaged DNA molecules in film for samples deposited on (a) glass, and (b) on tantalum as a function of thickness of DNA films for incident fluence of 10^{12} photons/cm².

Figure 3.

Dependence of the enhancement factor (EF) as an exponentially decaying function of the thickness of DNA films. The EF is derived by dividing the yield of damaged DNA on the tantalum substrate by that measured in DNA films on the glass, under the same experimental conditions.

Figure 4.
G-values for the loss of SC configuration of DNA induced by LEEs under a N₂ atmosphere as a function of film thickness.

Table 1

G_{LEE} (in nmol / J) for loss of SC obtained by various authors, under different conditions, along with the corrected G -values deduced from Eq. 5. The extent of hydration is expressed in terms of moles of water per mole of nucleotides, represented by Γ . For dried-frozen DNA samples Γ is considered to be 2.5 and for hydrated DNA $15 < \Gamma < 35^{13}$ (see Table 2). All data in this table were recorded with $\Gamma=2.5$ with the exception of those obtained in air at $\Gamma=20$.³³

References	Atmosphere	Film Thickness	G_{LEE}	G_P
Alizadeh et al. ³⁴	N ₂	10	227 ± 15	260 ± 50
Alizadeh et al. ³⁴	O ₂	10	415 ± 15	477 ± 38
Alizadeh et al. ⁴⁴	N ₂ O	10	737 ± 15	847 ± 67
Brun et al. ³³	Vacuum	10	400 ± 200	460 ± 230
Brun et al. ³³	Air	10	600 ± 200	690 ± 230
Cai et al. ³²	Vacuum	2	50 ± 16	295 ± 94

Table 2

G-values (in nmol / J) for formation of SSB in DNA by different type of radiation under various experimental conditions.

References	Radiation	Experiment	<i>G</i> -value
Ito et al. ¹³ (1993)	⁶⁰ Co γ -rays (1.17 MeV)	Dry DNA	56
		Hydrated DNA	120
Yokoya et al. ⁴⁸ (2002)	⁶⁰ Co γ -rays	Dry DNA	54 $\pm$ 9
		Hydrated DNA	72 $\pm$ 5
Yokoya et al. ⁴⁹ (2003)	α -particles (3.31 MeV)	Dry DNA	48 $\pm$ 4
		Hydrated DNA	60 $\pm$ 9
Purkayastha et al. ⁴⁵ (2006)	X-rays (70 keV)	Dry DNA	69 $\pm$ 14
		Hydrated DNA	54 $\pm$ 7
Cai et al. ³² (2005)	Al K $_{\alpha}$ X-rays (1486 eV)	Dry DNA	57 $\pm$ 1
Cai et al. ⁴⁷ (2005)	Al K $_{\alpha}$ X-rays	Dry DNA	62 $\pm$ 6
Yokoya et al. ⁴⁶ (2009)	Soft X-rays (2153 eV)	Dry DNA	43 $\pm$ 10
		Hydrated DNA	110 $\pm$ 12
Brun et al. ³³ (2009)	Al K $_{\alpha}$ X-rays	Dry DNA	44 $\pm$ 6
Brun et al. ³³ (2009)	Al K $_{\alpha}$ X-rays	Dry DNA	42 $\pm$ 6
Alizadeh et al. ⁴¹ (2011)	Al K $_{\alpha}$ X-rays	Dry DNA (N ₂)	60 $\pm$ 4
Alizadeh et al. ⁴¹ (2011)	Al K $_{\alpha}$ X-rays	Dry DNA (O ₂)	117 $\pm$ 8
Alizadeh et al. ⁴⁴ (2011)	Al K $_{\alpha}$ X-rays	Dry DNA (N ₂ O)	92 $\pm$ 13