Absolute Measurements of Radiation Damage in Nanometer Thick Films

Elahe Alizadeh* and Léon Sanche

Groupe en science des radiations, Département de médecine nucléaire et radiobiologie, Faculté de médecine et des sciences de la santé, Université de Sherbrooke, J1H 5N4, Sherbrooke, Canada

Abstract

We address the problem of absolute measurements of radiation damage in films of nanometer thicknesses. Thin films of DNA (~ 2–160nm) are deposited onto glass substrates and irradiated with varying doses of 1.5 keV X-rays under dry N₂ at atmospheric pressure and room temperature. For each different thickness, the damage is assessed by measuring the loss of the supercoiled configuration as a function of incident photon fluence. From the exposure curves, the *G*-values are deduced, assuming that X-ray photons interacting with DNA, deposit all of their energy in the film. The results show that the *G*-value (i.e., damage per unit of deposited energy) increases with film thickness and reaches a plateau at 30±5 nm. This thickness dependence provides a correction factor to estimate the actual *G*-value for films with thicknesses below 30nm thickness. Thus, the absolute values of damage can be compared with that of films of any thickness under different experimental conditions.

Keywords

Radiation damage; *G*-values; Thin film; Soft X-rays

1. Introduction

Questions related to the accurate quantification of ionizing radiation damage in materials of various chemical compositions and structures are of central importance to the radiation sciences. High-energy particles and photons can deposit a considerable amount of energy in irradiated matter that leads to damage and induces measurable changes in the optical, electrical and structural properties of organic and non-organic materials. These modifications can serve to evaluate the energy deposited or the dose absorbed in the target.
1,2,3,4,5

In the field of radiation detectors, efforts are devoted to explore the possibility of using various films for radiation detection and dosimetry. Estimates of the radiation sensitivity of thin layers of metal oxides, semiconductors, inorganic polymers and biopolymers have shown that films with thicknesses of the order of nanometers are generally more sensitive

*Corresponding Author: Elahe.Alizadeh@USherbrooke.ca; Fax: +1 819 564 5442; Tel: +1 819 346 1110 (15863).

per unit mass to high-energy radiation than thicker ones.^{6,7,8,9} Because very thin films of plastic scintillators can be fabricated, it is easy to provide a detector that is thin compared to the range of even weakly penetrating particles such as heavy ions. Thus, thin films can be useful transmission detectors for photons and alpha particles even when the energy deposited is relatively small. Additionally, in most applications when detectors with extremely high-radiation tolerance and fast response are required, reducing the thickness of semi-conductors provides an easy way to make more radiation tolerant detectors by reducing severe trapping of free carriers by radiation-induced defects that leads to degradations in sensor performance.¹⁰ In microelectronics, radiation effects on superconductors have mostly been studied in the bulk of substrates,^{11,12} while in applications of high temperature superconductors, much research is concerned with the determination of the processes involved in radiation damage and factors affecting the sensitivity of thin films of these materials. In these experiments, the change in temperature (as the damage factor) with fluence versus radiation energy loss has been measured.¹³ In the development of far-ultraviolet filters, radiation damage to optical materials has also been measured in the thick- and thin-film formats.¹⁴ Changes in transmittance or reflectance induced by radiation were found to depend on film thickness.¹⁴ Therefore, film thickness can change radiation damage and the energy deposited by modifying the molecular structure of the material and the energy deposition process.

In addition to studies in inanimate materials, particular interest has been given recently to radiation damage occurring in nanometer-thick films of biological molecules.^{15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30} Among these, irradiated DNA films of nanoscale thicknesses have received considerable attention owing to the vital importance of this target in radiobiology and radiotherapy.^{22,23,24,25,26,27,28,29,30} While many investigations have focused on the indirect effects of radiation using dilute aqueous solutions containing DNA,^{24,31,32} the direct effect has often been determined by irradiating nanoscale films of DNA with short-range particles or photons that penetrate less than a μm .^{18,20,21,23,30,33,34} This is particularly true for soft X-rays,^{30,34} 1 eV – 10 keV electrons^{19,20,21,26,29,35,36,37,38} and 10 eV – 1 MeV ions,^{28,39,40,41} which possess attenuation or thermalization distances of the order of 10 to 1000 nm in biological materials.^{42,43} Thus, with such radiation, the major emphasis for studies of the direct damage of ionizing radiation is placed on radiation tracks of nanometer lengths.

This situation is often met in the analysis of organic and biomolecular matter in the form of thin films with X-ray photoelectron spectroscopy (XPS) instruments.^{44,45,46} In such devices, the sample is usually irradiated with Al K α 1.5 keV photons. When it is deposited on a metal substrate, the thicknesses are usually smaller than a micron to reduce surface potentials created by film charging. In radiobiologically related studies, such X-rays are of particular interest owing to their high biological effectiveness. Compared to higher energy photons, soft X-rays produce lower energy primary electrons, with a correspondingly higher linear energy transfer (LET).⁴⁷ Additionally, basic radiobiological data obtained with soft X-rays has been useful for testing models of radiation damage in biological systems, such as inducing chemical modification in dry DNA, which revealed reactions pathways leading to base damage and strand breaks.^{16,40,41,44} Following the studies on the biological effectiveness of soft X-rays, some other studies showed that the sizes of the most sensitive

target volumes in radiation biology are of the order of a few nanometres.^{48,49,50} Thus, to explain the effect of ionizing radiation on biological systems in detail, the interactions of primary radiation and its resultant lower-energy secondaries must be considered in nanoscale volumes. As the main propose of nanodosimetry, such studies try to describe the biological effects of ionising radiation through evaluating its energy deposition in the targets with volumes of a few nanometres, which are comparable to the size of the DNA.⁵¹

In macroscopic systems, the amount of a specific type of damage imparted by radiation can be quantified on an absolute scale by measuring the G -value, defined as the number of moles of a given product per joule of radiation energy absorbed. It depends on the energy and type of radiation and provides absolute values so as to enable comparison among various studies. However, in the case of nanometer thick films below a certain thickness particles produced by the initial interaction escape from the film before depositing all of their energy.⁵² Thus, the G -values are underestimated. Measurements are useful for films of a given thickness, but without absolute values, it is difficult to make comparison between the effectiveness of different type of radiations on different targets.

Herein, we present measurements of G -values as a function of film thickness. We chose as a target plasmid DNA films of 2–160 nm average thickness deposited on a glass substrate and as a radiation source Al K_{α} X-rays of 1.5 keV. Such a source is commonly used in XPS analysis. The damage to plasmid DNA is measured as the decrease of the supercoiled configuration of the plasmid population upon irradiation. Thus, the corresponding G -value does not represent total damage to DNA, but only that modifying the supercoiled configuration such as strand breaks and cross-links. It is shown from our results that the calculated G -values for damage induced by soft X-rays measured in thin films of DNA become unreliable below a critical thickness of 30 ± 5 nm. From the film thickness dependence of the yields of damage, a factor can be obtained to correct G -values of too thin films. The G -values measured for nanometer thick films can therefore be extrapolated to those of bulk material (thick films) and hence provide a universal quantity for comparison to any other types of target or radiation.

2. Experimental Methods

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) with pH 8. Prior to use, DNA was cleaned of TE by applying a home-made microcolumn of Sephadex G-50 resin on a bed of glass beads, to remove the small molecules of salts from a solution.^{53,54} The concentration of DNA in the filtered solution was then measured spectrophotometrically from its absorbance at 260 nm, assuming a molar absorption coefficient of 5.3×10^7 L.mol^{–1}.cm^{–1} at pH 7.0 for DNA.⁵⁵ DNA solutions were then diluted in double distilled water (ddH₂O) to obtain the different concentrations of DNA required to make DNA films of variable thicknesses.

An specific volume (V) of plasmid solution in nanopure water with a known concentration was spread out on a chemically cleaned glass substrate, and then freeze dried (lyophilized) at -70°C under a pressure of $\sim 0.1\text{--}0.4$ mPa for about two hours. The dried sample had a ring shape with an average radius (r). Taking the density of $\rho = 1.71$ g/cm³ for plasmid extracted from *E. coli*,⁵⁶ and applying the Eq. 1, the average thicknesses of different groups of DNA films were determined by

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

Here, m_{DNA} is the mass of DNA in each sample, S is the area and t is the mean thickness of the film, respectively. 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 .

2.2. Experimental Setup and Irradiation Conditions

The experiments were performed with an apparatus recently developed by Alizadeh et al.⁵⁷ which has been previously applied to similar studies on thin DNA films of constant thickness. The method has been described in detail elsewhere.⁵⁸ Here, only a brief description is given. The Al K α X-rays (with energy about 1.5 keV) are generated from a cold-cathode source constructed according to on the original design of Hoshi et al.⁵⁹ A plasma discharge with 5.5 mA current is formed between the cathode and an aluminum foil target in a small stainless steel chamber. Aluminum characteristic K α X-rays are produced by electron bombardment of the target and travel through a flight tube continuously flushed with helium gas at atmospheric pressure. X-rays then pass through a thin foil of Mylar to enter a small chamber filled by dry N₂ at atmospheric pressure with 99% stated purity and no humidity, as monitored by a hygrometer in the irradiation chamber. Within this chamber the freeze-dried DNA films are deposited in front of the source, on glass substrates, where they are exposed to X-rays of varying fluence. For each group of films, two films serve as controls. These latter films are kept under the same experimental conditions as the irradiated samples, and recovered, but are not irradiated with X-rays.

2.3. Quantification of the Yields by Agarose Gel Electrophoresis

In the present experiments, the samples were immediately retrieved from the chamber after irradiation, and dissolved in TE buffer from the glass surfaces with 95–98% efficiency. The fractions of various forms induced in DNA by irradiation were then analyzed 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. DNA damage was assessed by measuring the fractions of undamaged plasmid in the supercoiled form (SC) and modified DNA in the relaxed or ‘nicked circular’ form (C) from single strand break (SSB). Before irradiation, about 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 $\times$ SYBR[®] Green I (Molecular Probes[™]) before 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 $\times$ concentration SYBR Green I. After electrophoresis, gels were scanned with the Typhoon-Trio laser scanner (GE Healthcare) using blue fluorescence mode at an excitation wavelength of 488 nm and filter type 520 BP 40. The 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 the nicked circular and linear configurations. For pGEM-3Zf(−) plasmids irradiated in this work, correction factor of 1.4 was determined after quantification by ImageQuant. The number of incident photons on the samples was determined using GAFCHROMIC[®] HD-810 dosimetry films. A detailed description of the calculations and the calibration of the sensitivity of the films can be found in Ref. 57.

2.4. Calculation of X-ray 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 atomic composition of DNA and μ/ρ of individual atoms.⁶⁰ For each known thickness, the dose-response curves (i.e., percentage loss of SC DNA as a function of incident photon fluence) were plotted for fluences from zero to $35 \times 10^{11} \text{ photons/cm}^2$. Each of these dose-responses curves required measurements on at least 3 samples at each 4–5 different photon fluences. These measurements were repeated at different film thicknesses for a total of 140 analyses of DNA damage by agarose gel electrophoresis. The number of damaged DNA molecules for a given photon fluence (Φ) was found via $D = |SC| \times \Phi \times N_{DNA}$, where N_{DNA} is the number of DNA molecules in each sample and $|SC|$ the absolute value of the slope of the dose response curve for the loss of the SC configuration. The number of absorbed photons in the DNA film was calculated by

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

The G -values can be expressed in two different units, $D/100\text{eV}$ and nmol/J or $\mu\text{mol/J}$, where D represents, in our case, one damaged DNA molecule. From our results, G -values for Al K α X-rays of 1.5 keV were calculated using

$$G = \frac{D_{Gl}}{X_{Abs} \times 1486\text{eV}} \times 100\text{eV} \quad (3)$$

This Equation gives G -values in $D/100 \text{ eV}$, where $1 D/100 \text{ eV} = 103 \text{ nmol/J}$. G -values are calculated within 20% error, which mostly arises from the uncertainty on S (i.e., the area of the DNA film), as well as the concentration of DNA in the solution that is measured spectrophotometrically.

3. Results and Discussion

Figure 1 shows the exposure-response curves for loss of the SC form of DNA at thicknesses of 2, 10, 30 and 150 nm. The slopes (ΔSC) of the linear-least-square fits of respective exposure curves, indicated on each panel of the figure, represent the fluence-normalized yields of induced damage for each film thickness. Only a small portion of the incident photon fluence indicated on the X-axis of Fig. 1 interacts with the DNA forming the film. In the thinnest films (~ 2 nm), more than 99.6% of the photons pass through the DNA to enter the glass substrate, where they are absorbed. For the thickest film in this work (~ 160 nm), almost 3% of the incident photons interact with the DNA (i.e., 97% reach to the substrate). Figure 2 exhibits the dependence of the number of absorbed photons versus the thickness of DNA films for an incident fluence of 10^{12} photons/cm². The absorbance obeys a linear function with film thickness, but according to the Beer-Lambert law this graph should have an exponential function behavior. However, for the range of film thicknesses in our experiments, the small absorption of the X-rays in DNA increases linearly with film thickness within experimental errors.

Consequently, as the film thickness increases, the number of damaged molecules is expected to increase linearly. Figure 3 represents the variation in the number of damaged DNA molecules induced in the plasmid as a function of film thickness. It shows that, within the estimated errors, the number of damaged DNA molecules increases linearly with film thickness, from ~ 20 to 160 nm. Below 20 nm the yield of damage molecules lies below the linear fit. This behavior affects the calculated G -values shown in Figure 4. The G -values for X-rays increase rapidly with increasing thickness up to around 30 ± 5 nm, after which they remain roughly constant at 98 ± 20 nmol/J. This behavior is expected, since according to Eq. 3, when the number of absorbed photons and the number of damaged molecules both increase linearly, the G -value must remain constant. On the other hand, when the DNA films are too thin the G -values for 1.5 keV X-rays are underestimated.

It is well known that the dominant process in the interaction of Al K α X-rays with DNA is photoabsorption, which results in the ejection of a photoelectron of about 1.5 keV, with a range of about 80 nm, or two Auger electrons⁴⁴ with dominant energies of 0.96 and 0.52 keV⁶¹ and ranges of about 36 and 15 nm in the DNA films, respectively.⁶² Most of these electrons transfer little momentum to DNA. Under this condition their probability of energy transfer to the molecule is similar to that of photons.⁶³ Thus, even photoelectrons and most Auger electrons produced in the middle of a 30 nm film of DNA can be transmitted through the 15 nm of the layer, if they are emitted perpendicular to the film surface. However, according to the results of Fig. 4 beyond thicknesses of 30 ± 5 nm, most of the photoelectrons and Auger electrons seem to deposit most of their energy in the DNA film. The portion that escapes into the surrounding N₂ atmosphere or inside the substrate, with a significant amount of energy is negligible within the quoted errors. This is probably due to the angular distribution of the emitted electrons. At smaller thicknesses (< 30 nm) more energy is expected to escape outside the film. This phenomenon appears sufficient to account for the smaller G -values measured at low thicknesses, such as ~ 65 nmol/J for 10 nm films. As a general rule, with increasing the thickness, a higher proportion of photoelectrons and Auger electrons interact with the film. About 20% of the energy of these

primary electrons is deposited into vibrations and electronic excitation.⁶⁴ The remaining energy flows into ionization as kinetic energy of secondary electrons and potential energy of the DNA radical cations, with the largest portion going to secondary electrons.^{52,64,65} Many of these processes as well as those created by the secondary electrons can dissociate chemical bonds within DNA and induce strand breaks and other forms of damage.^{64,66} Energy also escapes from the film as low energy electromagnetic radiation and vibration, but portion of the energy transferred outside the film is expected to be constant for the range of thicknesses investigated. Furthermore, these low energy processes may not cause significant damage to DNA. We therefore conclude that, according to Fig. 4, beyond 30 ± 5 nm thickness essentially all the energy of the primary and secondary electrons is absorbed in the films, causing the G -value to be constant for soft X-rays.

Knowing the G -value at the plateau in Fig. 4, i.e., 98 ± 20 nmol/J under N_2 atmosphere, allows calculating a factor to find the difference between the most reliable G -value and those measured at smaller thicknesses. This factor can be calculated by

$$f = \frac{G_P - G_t}{G_P} \quad (4)$$

where f is the fraction of actual damage induced in the films of thickness (t) and G_P and G_t

are 1 the G -values measured at the plateau and at t , respectively. Alternatively, $CF = \frac{1}{1-f}$ can be considered as a correction coefficient for finding the real G -values, under different environmental conditions, at each specific thickness. Table 1 lists the G -values obtained previously with the same technique, but under different environmental conditions.^{57,67,68,69,70} The corrected G -values deduced from Eq. (4) and Fig. 4 are given in the last column. They are almost 33% higher than the ones measured in previous experiments for 10 nm films. The data recorded at large thicknesses (i.e., 10 and 20 μ m) given in Table 1 are from the work of Cai. et al.;^{69,70} they were obtained by the same technique under vacuum conditions. We have shown previously that damage to DNA induced by 1.5 keV photons was the same in vacuum as in a N_2 atmosphere, i.e., gaseous N_2 at STP does not promote radiation damage to DNA.⁵⁷ We can therefore compare our results to those of Cai. et al.^{65,66}. As shown in the last two lines of Table 1, within standard errors, the large thickness G -values, i.e., 80 ± 1 and 99 ± 14 nmol/J,^{69,70} are in good agreement with our plateau value, i.e., 98 ± 20 nmol/J. This agreement confirms that by increasing the thickness much beyond 30 nm, the G -values remain constant at G_P .

4. Conclusions

In this work, damage to plasmid DNA films was measured in the form of decrease in the SC population and the G -values for loss of the SC form, induced by 1.5 keV photons, were determined for film thicknesses ranging from ~ 2 nm to ~ 160 nm. Absorption of soft X-rays in the DNA molecules results in the generation of primary electrons almost exclusively by the photoelectric effect. Thus, the investigation of X-ray photons interaction is essentially

equivalent to that of electrons since the energy of the photons is transferred to primary electrons. The energy of most of these electrons ranges from 0.52 to 1.5 keV, which results in short penetration distances (15–80 nm) in DNA films. Despite these short ranges, when the film thickness is too small, primary and possibly secondary electrons can transmit through the thin film without depositing all of their energy. Owing to this phenomenon, the calculated G -values for damage in thin films of DNA become unreliable below a critical thickness. Our results show that previously measured G -values for thin DNA films were too small by a factor of $\sim 33\%$.

From comparison of previous data and new results from the present work, we found that (1) the G -values for DNA damage induced by soft X-rays measured in films with average thicknesses less than 30 ± 5 nm may be too low; (2) we can define a function $G(t)$, which describes the behaviour of the G -value as a function of thickness and allows to correct G -values of too thin films; (3) $G(t)$ reaches a plateau that provides a more reliable G -value; (4) because of the universality of G -values, this study permits comparison of the absolute damage to the SC configuration imparted to nanometer-thick plasmid DNA films by 1.5 keV photons under a nitrogen atmosphere to that obtained in larger systems by the same or other types of radiations. The present method should be applicable to experiments with other materials and radiations, when the energy absorbed from the initial interactions is not all deposited in an irradiated thin film.

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Fig. 1.

Exposure-response curves for loss of SC form of DNA at thicknesses of 2, 10, 30 and 150 nm. Each point represents the mean of the percentage yields obtained from three DNA films irradiated by soft X-rays. The error bars represent the standard deviation of the mean. SC is the slope of each curve.

Fig. 2.
Dependence of the number of absorbed X-ray photons as a function of thickness of DNA films for an incident fluence of 10^{12} photons/cm².

Fig. 3. Number of damaged molecules as a function of thickness of DNA films deposited on glass for an incident fluence of 10^{12} photons/cm².

Fig. 4.
The G -values for loss of SC form of DNA as a function of thickness of DNA films for Al K_{α} soft X-ray irradiation under a N_2 atmosphere.

Table 1

Al K α X-ray G -values obtained under different conditions along with the corrected G -values deduced from Fig. 4 and Eq. (4) with G_t taken at the specific thickness $t = 10$ nm. Data previously recorded at large thicknesses (10 and 20 μm) can be compared with data in this work, because of the similarity between G -values obtained under vacuum and N $_2$ atmospheres.⁵⁷

References	Atmosphere	Film Thickness (nm)	G_t (nmol/J)	G_P (nmol/J)
Alizadeh et al. ⁵⁷	N $_2$	10	65 ± 6	98 ± 20
Alizadeh et al. ⁵⁷	O $_2$	10	124 ± 9	187 ± 14
Alizadeh et al. ⁶⁷	N $_2$ O	10	103 ± 8	155 ± 12
Brun et al. ⁶⁸	Vacuum	10	44 ± 6	66 ± 9
Brun et al. ⁶⁸	Air	10	42 ± 6	63 ± 9
Cai et al. ⁶⁹	Vacuum	20,000	80 ± 1	98 ± 20
Cai et al. ⁷⁰	Vacuum	10,000	99 ± 14	98 ± 20