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Version: [\[not recorded\]](#)

Link(s) to article on publisher's website:
<http://dx.doi.org/doi:10.1063/1.2961027>

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X-ray induced damage in DNA monitored by X-ray photoelectron spectroscopy

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(Received 27 March 2008; accepted 27 June 2008; published online 11 August 2008)

In this work, the chemical changes in calf thymus DNA samples were analyzed by X-ray photoelectron spectroscopy (XPS). The DNA samples were irradiated for over 5 h and spectra were taken repeatedly every 30 min. In this approach the X-ray beam both damages and probes the samples. In most cases, XPS spectra have complex shapes due to contributions of C, N, and O atoms bonded at several different sites. We show that from a comparative analysis of the modification in XPS line shapes of the C 1s, O 1s, N 1s, and P 2p peaks, one can gain insight into a number of reaction pathways leading to radiation damage to DNA. © 2008 American Institute of Physics.

[DOI: [10.1063/1.2961027](https://doi.org/10.1063/1.2961027)]

I. INTRODUCTION

When ionizing radiations, from natural background radiation or derived from diagnostic and therapeutic devices (e.g., X rays, radiotherapy, and positron emission tomography), interact with biological tissue, it can produce a range of structural and chemical modifications of the DNA helix. Of these, double-strand breaks (DSB), where both strands of the helix are broken within a few base pairs, can lead to lasting damage via the production of chromosome aberrations, mutations, and ultimately cell death.

Generally radiation damage to DNA can be classified either as indirect or direct type damage.^{1,2} The first type is due to the attack of DNA by highly reactive radicals that are products of ionizations in the surrounding medium. However, the mobility of formed radicals is not significant and, additionally, the presence of radical scavengers in cells often protects the DNA molecule. Therefore, the importance of direct-type damage is substantial and *in vivo* has been estimated to contribute 40%–50% of the DNA damage.³ The term “direct-type damage” refers to the direct ionization of DNA or transfer of electrons or holes to DNA from its solvent shell.

Hence, in order to understand the mechanism of radiation damage, it is essential to understand the details of the interaction of different types of radiation with the constituent cellular molecules (DNA itself and DNA-protein complexes). Chemical modifications in dry DNA induced by X rays have been analyzed using agarose gel electrophoresis, which has shown the formation of single-strand break and DSB and crosslinks between DNA molecules (for the most recent paper see Ref. 4) and base lesions.⁵

The present work is part of an ongoing effort to deter-

mine the details of the mechanism by which ionizing radiation directly damages DNA. Here radiation-induced chemical modifications in DNA films are monitored by X-ray photoelectron spectroscopy (XPS). This technique not only complements traditional biochemical analysis [e.g., gel electrophoresis or high performance liquid chromatography (HPLC)] but also provides *in situ* results with and without postirradiation treatment. As one of the most powerful methods of surface analysis, it allows quantitative elemental analysis of thin films and provides information about the chemical transformation of functional groups. Moreover, the XPS method is able to characterize the elements and molecules present in the top 20–100 Å of a surface⁶ with a detection limit as low as a few ng/cm².⁷

In our approach the X-ray beam both damages and probes DNA samples. Due to the intricate molecular makeup of nucleic acids, the resulting XPS spectra are correspondingly complex. However, when this technique is adapted to crystals of biological macromolecules, it offers distinct advantages compared to studies in solution: such crystals contain higher concentration of the macromolecules and have a relatively low solvent content.

II. EXPERIMENTAL SETUP

A. Materials and sample preparation

Calf thymus doubled stranded DNA (the molecular weight is reported to be between 10×10^6 and 15×10^6 Da, sodium chloride) and nucleobases (adenine, cytosine, guanine, and thymine) were all purchased from Sigma-Aldrich and used without further purification. An aqueous DNA solution (1 mg/ml) was deposited onto silicon substrates (Compart Technology Ltd.) over an area of 0.6 cm² and dried in air at room temperature. Silicon substrates were cleaned prior to each deposition of DNA in piranha solution, i.e., a 3:7 mixture of H₂O₂ and H₂SO₄ (piranha solution must

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be handled with care due to its strongly oxidizing reaction), in order to remove all traces of organic materials at the surface. Subsequently, the substrates were rinsed with ultrapure water from a Millipore system and dried using a nitrogen stream.

The nucleobases were loaded into an oven and thermally evaporated onto the surface of MoS₂ crystals (Wards Foundation). The evaporation temperatures were 105 °C for thymine, 135 °C for cytosine, 150 °C for adenine, and 275 °C for guanine. The substrate was cleaned before each deposition by resistive heating to 700 °C.

B. XPS measurements

XPS measurements were performed using commercial XPS systems at the Open University (OU), Milton Keynes (Kratos XSAM 800) and in Sherbrooke (Perkin Elmer ESCA 4.0) equipped with a dual anode x-ray gun, a concentric hemispherical electron energy analyzer, and a channeltron detector. The OU source was a magnesium *K*α X-ray beam with a resolution of 0.7 eV. In our experiments, performed at a base pressure of 2×10^{-9} mbar, the x-ray power was kept relatively low (260 W at 13 kV) to minimize sample heating. The angle of incidence of the X-ray beam with the sample normal was about 60°. XPS spectra were recorded in the fixed analyzer transmission (FAT) mode with a pass energy of 20 eV and with energy steps of 0.1 eV. The magnification of the analyzer in the FAT mode was selected to collect photoelectrons from an area of approximately 4 mm². Due to charging effects during X-ray irradiation of the powdered organic samples, the energy axis of the XPS spectra is usually shifted to make the C 1s binding energy line equal to 285 eV, a standard hydrocarbon energy of C—H and C—C bonds.⁸ However, in the present studies only very weak charging features were observed (e.g., low binding energy tails), most likely because sufficiently thin DNA films were prepared on grounded substrates using silver conductive paint.

In the case of DNA, peak areas and elemental composition were calculated with the commercial XPS analysis software CASAXPS.⁹ This software also served to fit peaks to the high resolution spectra for all elements, using a convolution of Gaussian line shape. All XPS spectra were taken with acquisition times that are a compromise between the signal-to-noise ratio in the spectra and the rate of radiation-induced decomposition. Every scan for each element region (carbon, nitrogen, oxygen, and phosphorous) was recorded for over 30 min. In order to monitor changes on sample surfaces, the spectra were taken repeatedly for over 5 h. At least ten cycles were recorded for each element region of a DNA sample.

The high vacuum multifunctional apparatus used in Sherbrooke to characterize the surface of nucleobases is described elsewhere.¹⁰ Briefly, the X-ray source (15 kV, 400 W) is composed of a magnesium anode and an Al window was used to characterize the surface of nucleobases. The X-ray beam was incident at 72° relative to the sample surface, and the hemispherical electron energy analyzer was positioned normal to the surface. The pass energies of the elec-

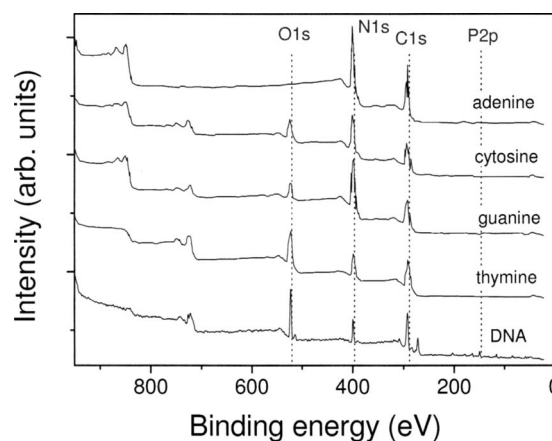


FIG. 1. XPS survey spectra of all DNA nucleobases: adenine, cytosine, guanine, thymine, and a DNA-calf thymus sample.

tron energy analyzer were set at 2.95 and 29.35 eV, which correspond to XPS spectral resolutions of $\Delta E = 0.76$ eV and $\Delta E = 1.05$ eV, respectively, as estimated from the full width at half maximum (FWHM) of the Ag *3d*_{5/2} spectrum. Additionally, for the purpose of spectral analysis, the resolution has been improved to 0.3 eV by deconvoluting the width of the Mg *K*α radiation.¹¹ Spectra of all detected element regions were accumulated for 2 h; no chemical changes in nucleobase samples occurred during that acquisition time.

III. RESULTS AND DISCUSSION

A. XPS characterization of nucleobases

The survey spectra of all nucleobase films are shown in Fig. 1. These spectra reveal the C 1s, N 1s, and O 1s photoelectron lines (H is not observable by XPS) and the Auger electron lines. Each 1s peak is accompanied by a satellite peak located at a higher binding energy and originated from shake-up and shake-off transition¹² and plasmon losses. In contrast to other nucleobases the adenine spectrum shows no feature around 520 eV attributed to the O 1s core-level photoemission, which is consistent with the known structure of this base, which contains only C, N, and H atoms.

Using the coefficients of elemental sensitivity from the literature,⁸ the film composition was determined for four nucleobases. The atomic concentrations of each element observed in Fig. 1 are presented as percentages in Table I. For comparison, the atomic concentrations calculated from the

TABLE I. The atomic concentrations of C, O, and N in the films of nucleotide bases determined from the XPS data compared with that expected from their chemical formula.

Compound	Atomic concentrations derived from the XPS data			Atomic concentrations derived from the chemical formula of the compound		
	C	O	N	C	O	N
Adenine	49.55	0	50.45	50	0	50
Cytosine	49.66	13.18	37.16	50	12.5	37.5
Guanine	44.38	9.77	45.86	45.45	9.09	45.45
Thymine	55.56	22.66	21.78	55.55	22.22	22.22

chemical formula of the compound are also included. The theoretical compositions were predicted from the known structure of each nucleobase, but due to the impossibility of hydrogen detection by XPS, H atoms were excluded from these considerations. The XPS-determined composition of nucleobases is in extremely good agreement with the chemical formula of the compound and also agrees with previously reported data.¹³ The mean square deviation of the measured composition from the chemical values is below 5%, indicating that neither molecular decomposition occurred during the thermal evaporation of nucleobase films nor was there evidence for any contaminations on the surface (e.g., hydrocarbons from pump oils). Due to possible carbon contamination in the sample,¹⁴ the C 1s spectra should be considered with care. An independent measurement of carbon contamination in the apparatus was performed. A silicon substrate cleaned by Ar ion sputtering was kept under vacuum condition for over 24 h. After this period of time a survey spectrum was recorded, which showed an increase in the C 1s peak of about 5% compared to a spectrum taken immediately after ion sputtering. However, the same experiment was performed for a DNA sample. The C 1s spectra and also N 1s, O 1s, and P 2p do not show any changes for DNA kept in ultrahigh vacuum conditions after 6 h.

Previous studies reported 1s core electron binding energies for selected elements of different species, i.e., C 1s spectra were recorded for condensed phase of all nucleobases,^{13,15} N 1s for thymine^{15,16} and guanine,¹⁵ and O 1s for cytosine¹⁶ and thymine.^{15,16} In the present work, high resolution C 1s spectra of DNA nucleobases are shown in Fig. 2 and exhibit a complicated structure due to contributions from different molecular species containing carbon atoms. Using results from previous studies on nucleobases, nucleosides, nucleotides, and oligos,¹³ the major peaks in the spectra here can be uniquely assigned to the carbon species present in our samples. The following species are involved: hydrocarbons (C—C, C—H), carbon bound to nitrogen (C—N, N—C—N), amide carbon (N—C=O), and urea carbon [N—C(=O)—N] with characteristic binding energies of approximately 285, 286–287, 288, and 289 eV, respectively. As can be seen from the spectra in Fig. 2(a), the different nucleobases could be distinguished based on their characteristic C 1s spectra. For example, the peak at 285 eV is assigned to a hydrocarbon group and is observed for all nucleobases; however the relative intensity of this peak with respect to other peaks is different for all the nucleobases. Adenine has a double ring structure that contains carbon, nitrogen, and hydrogen atoms; therefore it does not show any signal for either urea or amide groups. The peak assigned to the presence of the urea group also does not appear in the guanine spectrum, which is in agreement with the molecular structure of this compound.

The N 1s spectra [Fig. 2(b)] reveal a two-component structure for all DNA nucleobases, except thymine but the separation between the components is lower than those in C 1s. The N 1s XPS spectra can be divided into two regions above 400 eV and around 398–399 eV. A higher energy peak is attributed to amino N sites that connect with single bonds. This agrees with XPS studies for several amino acids

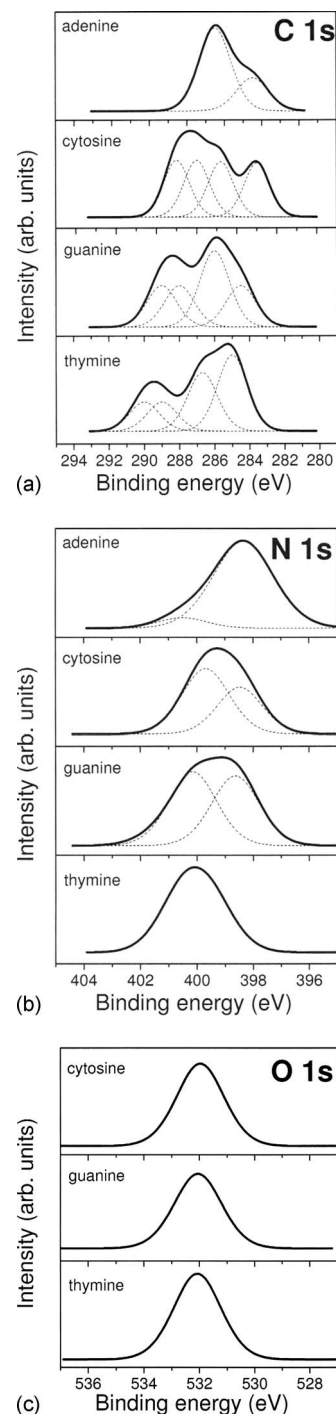


FIG. 2. High resolution XPS spectra of the (a) C 1s, (b) N 1s, and (c) O 1s regions for all nucleobases. The 1s peaks were deconvoluted using linear superposition of a few Gaussian components with description given in the text.

containing the NH_2 group.¹⁷ A peak at the lower binding energy can be attributed to imino species that include a double $\text{N}=\text{C}$ bond.¹⁸ This assignment is also in very good agreement with the density functional theory calculations of inner shell ionization energies of imino and amino sites in cytosine and cytidine.¹⁹

In the case of the O 1s spectra for many pyrimidine and purine molecules, a single peak is resolved at a binding energy between 531 and 532 eV (Refs. 13 and 15) assigned to

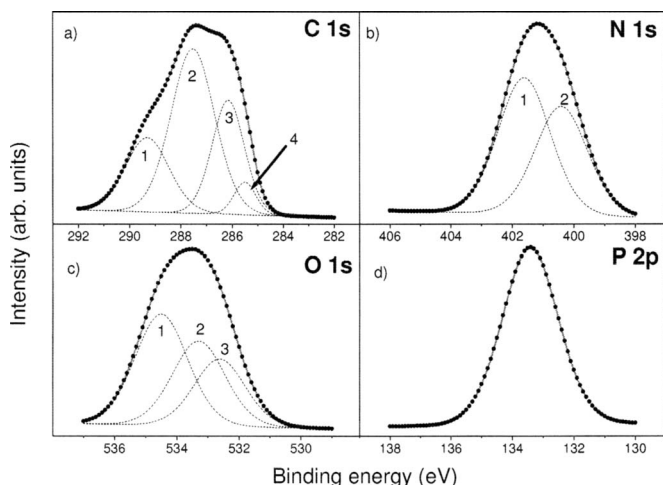


FIG. 3. High resolution XPS spectra of C 1s, N 1s, O 1s, and P 2p regions for a DNA sample: experimental data (solid curve) and fitting results (thin curve). The 1s peaks were deconvoluted using linear superposition of a few Gaussian components. A single Gaussian peak has a FWHM of 2 eV. Peak labeling: (a) C 1s, urea (peak 1), amide (2), alcohol/cyclic ether/ carbon bond to nitrogen (3), and hydrocarbon (4); (b) N 1s, amino (1) and imino (2) sites; (c) O 1s, alcohol (1), carboxyl/carbamido groups (2), and the phosphate group (3); (d) P 2p, the phosphate group.

the C=O bond [Fig. 2(c)]. As expected, we do not see an O 1s XPS spectrum in adenine since adenine does not contain oxygen.

B. XPS characterization of DNA

DNA is made up of the different nucleobases, each attached to a deoxyribose sugar and a phosphate group. The survey spectrum of calf thymus DNA was primarily used to control an effectiveness of sample coverage and to monitor for the presence of any contaminants (Fig. 1, the lowest panel). Generally, the N and P elements are unaffected by surface contamination during sample preparation and handling; thus their existence indicates adsorption of the DNA sample onto the substrate.²⁰ In addition to four principal elements in a film (C, N, O, and P), a peak is observed at the binding energy of 272 eV attributed to the ejection of photoelectrons from Cl 1s due to the presence of salt in the sample. No peak was observed in the region characteristic of the silicon substrate (99.7 eV).

High resolution spectra recorded for the C 1s, N 1s, O 1s, and P 2p regions are presented in Fig. 3. In the absence of *ab initio* calculations, the number of peaks was chosen to fit each elemental region corresponding to particular species. A typical FWHM value of fitted peaks is typically between 1 and 2 eV for polymerlike materials. In the present work, due to a nonmonochromatic X-ray source used, the FWHM of each fitted peak in high resolution spectra of DNA elements was constrained to the value of 2 eV.

The assignment of the deconvoluted C 1s spectral peaks can be carried out using known substituent effects of core electron binding energies.²¹ In Fig. 3, the major peaks expected from nucleobases are present in the C 1s feature of DNA, i.e., urea (peak 1), amide (2), carbon bond to nitrogen (3), and hydrocarbon (4). However their intensities vary because of the number of different bases contained in the

sample and the number of sugar units. With the addition of deoxyribose to a base, new contributions are expected to be observed at a binding energy of around 286.5 eV [peak 3 in Fig. 3(a)], which can be attributed to the carbon atom attached to oxygen forming C—O—C and the C—OH bonds in the sugar unit. The binding energy of photoelectrons ejected from these species corresponds to a value of electron binding energy from a C—N bond. Therefore peak 3 in Fig. 3(a) includes contributions from a deoxyribose sugar, i.e., alcohol and cyclic ether, and from nucleobase, i.e., C—N bonds. Previously reported C 1s spectra from single stranded DNA oligonucleotides^{20,22} are slightly different from those presented here, particularly with respect to the relative intensities of subpeaks. As shown in Fig. 2(a), each C 1s spectrum is characteristic for a specific nucleobase, depending which functional groups are present. The sequence in DNA varies for different samples; therefore discrepancies observed in spectra of DNA in different experiments may be explained by different nucleobase contents. For example, the intensity ratio of contributions in C 1s spectra of a single stranded DNA that contained only thymine molecules²⁰ differs from that observed for a single stranded DNA, which contains the mixture of nucleobases.²²

The principal N 1s core-level peak in Fig. 3(b) has a binding energy between 403 and 399 eV, consistent with published results for other DNA samples.^{20,22} Since neither the sugar nor phosphate groups contain nitrogen, no significant changes in comparison to nucleobase spectra are expected. However, due to nucleobase conjugation to a sugar via N-glycosidic bond, an increase in the intensity at a higher energy attributed to the amino site is observed. The change in the N 1s spectra between nucleobase, nucleoside, and nucleotide has been reported in previous experimental studies on uracil, uridine, and uridine monophosphate.¹³

The effect of adding sugar and phosphate groups to a nucleobase is also noticeable in the O 1s region.¹³ Previously reported XPS spectra for uracil, uridine, and uridine monophosphate have shown a shift in the O 1s peak structure, which was attributed to C=O from the two amides in the structure of nucleobases. The observed shift is due to the incorporation of a large amount of C—OH in the sugar residual. Additional broadening of the O 1s peak structure is caused by contributions from oxygen in the phosphate group.¹³ Thus three Gaussian peaks are fitted to our O 1s spectrum with peak maxima at 534.5, 533, and 532.5 assigned as C—OH bond, C=O bond, and oxygen in the phosphate group, respectively [Fig. 3(c)]. In the case of P 2p signal [Fig. 3(d)], an asymmetric shape is observed due to spin-orbit coupling, which leads to a splitting of the 2p phosphorus into two subpeaks with a 0.5 intensity ratio.

C. Compositional changes of DNA induced by X rays

To account for the radiation damage induced by X rays, a film of DNA has been irradiated with X rays continuously for about 5 h. Each of the XPS regions of interest was recorded successively with high resolution. Qualitative behavior can in most cases be deduced by visual comparison of the spectra before and after irradiation. For N 1s, O 1s, and P 2p

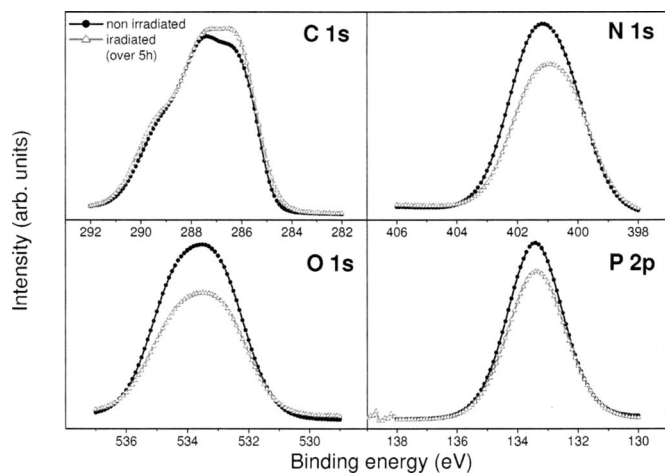


FIG. 4. High resolution XPS spectra of C 1s, N 1s, O 1s, and P 2p regions for pristine DNA samples. (closed circle) and after continuous 5 h exposure to x rays (open triangle).

presented in Fig. 4, a decrease in peak intensity for over 5 h of continuous X-ray irradiation is observed. This indicates that it is not the evaporation of molecules that is responsible for the observed changes in the XPS spectra but due to photon-induced decomposition accompanied by desorption of small fragments. The release of fragments from the surface is also called mass loss. However, in the case of C 1s a slight increase of a signal at 286 eV is recorded. Mass spectrometric studies on desorption of fragments during soft X-ray induced decomposition of amino acids showed that only low-molecular-mass gaseous species are detected.²³ This can be due to the fact that bigger fragments do not have enough kinetic energy in order to be desorbed. In the present studies on DNA damage, we assume that observed mass loss is due to desorption of small fragments containing N and O atoms, which can be released by simple bond cleavages, e.g., C—NH₂ and C=O bonds, respectively, while fragments that contain C atoms are mostly involved in ring structures. However, to our best knowledge no spectrometric studies on fragment desorption from DNA or nucleobases induced by X-ray have been reported.

To determine the chemical modifications caused by an irradiation period, the area under the main 1s and/or 2p peaks was determined for a DNA sample as a function of the irradiation time [Fig. 5(a)]. As shown in Fig. 5(a), the total concentration of oxygen in a DNA sample drops by about 30% for 2.5 h of irradiation and afterward remains relatively constant, whereas the concentrations of nitrogen and phosphorus atoms decrease only slightly over the entire period of irradiation. However, the relative amount of carbon increases slightly after 2 h of X-ray exposure.

Absolute areas for fitted peaks to each XPS line shape versus X-ray irradiation time are plotted in Figs. 5(b)–5(d). In the case of C 1s [Fig. 5(b)], the three peaks attributed to urea, amide, and hydrocarbon sites remain relatively constant, whereas the peak labeled 3 shows a pronounced increase. The components at 286–287 eV can be assigned either to alcohol (C—OH), cyclic ether (C—O—C), or imino groups. The rise of the signal identifies that a new product is formed as a result of X-ray exposure, which con-

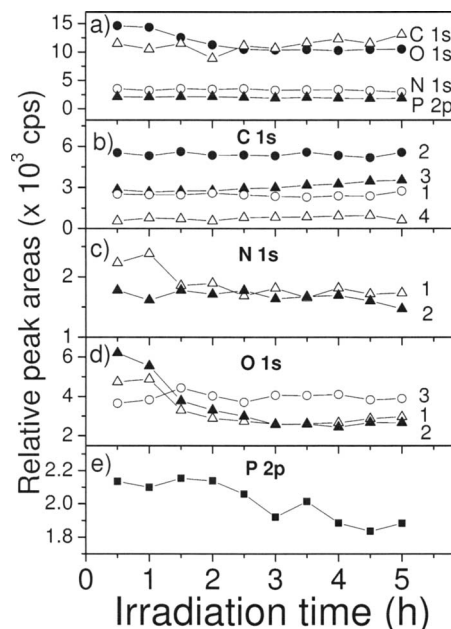


FIG. 5. (a) The total peak areas vs irradiation time for all elements in a DNA sample. Peak areas were recorded every 30 min for over a period of 5 h. [(b)–(e)] Time evolution of fitted Gaussian peaks from Fig. 3 for C 1s, N 1s, O 1s, and P 2p, respectively. Each point represents the average value obtained from spectra of at least three samples. The estimated error does not exceed 10%.

tains one of the above components. Radical formation, which is revealed under radiation exposure, may possibly explain the observed changes. A previous electron paramagnetic resonance study on degradation of thymine by X-ray irradiation showed the formation of a thymine allylic radical, which is created by H abstraction from a methyl group.²⁴ The authors suggested that thymine allylic radical can be a precursor of 5-formyl uracil and hydroxymethyl uracil, where the latter one contains a C—OH bond in its molecular structure. The formation of hydroxymethyl uracil in a DNA sample can also be expected in the present studies, but other products may be possibly generated; therefore, further studies are necessary.

The time evolution of areas for the two spectral features that we attribute to amino (peak 1) and imino (peak 2) sites in DNA nucleobases is presented in Fig. 5(c). The nitrogen in amino sites can exist as N bonds with two protons (the NH₂ group) and with a carbon atom in the deoxyribose through the N-glycosidic bond. The peak corresponding to the amino group drops rapidly after 1 h of X-ray irradiation. The decrease of the N 1s signal at a higher energy can be attributed to dehydrogenation of amino groups and/or to the breaking of the N-glycosidic bond. The latter process, which involves free unaltered base release, may cause the loss of genetic information and thus lead to mutations in the DNA sequence.²⁵ Moreover, base release under ionizing radiation is known to be correlated with DNA strand breaks; however the mechanism of possible pathways to form strand break is still a subject of many studies.²⁶ N loss is also observed at low binding energy attributed to the imino site; however this signal begins to decrease only after 4 h of continuous irradiation. After this time, the peak area for the imino group drops about 15% within 1 h [see Fig. 5(c)].

The peak areas for three components of O 1s as a function of irradiation time are presented in Fig. 5(d). The amount of oxygen in the phosphate group (peak 3) is constant, while peak areas labeled as 1 and 2, which are attributed to C—OH and C=O functional groups, respectively, decrease as a function of irradiation time. The stability of peak 3 is not too surprising since radiation damage to the backbone close to oxygen usually results in strand break via C—O bond scission, which does not lead to loss of material from the target. On the other hand, the C—OH group exists in the sugar moiety of DNA; thus, COH loss from the sample indicates the decomposition of a sugar molecule. X-ray induced ion desorption from deoxyribose sugar showed a rich fragmentation pattern, where mainly hydrocarbons and $C_xOH_x^+$ ($x=1, 2, \text{ and } 3$) ions were detected.²⁷ The C=O peak (peak 2) can be assigned to the carboxyl or carbamido groups, both present in the nucleobases. The loss of these species indicates the strong degradation of nucleobases of about 50% [Fig. 5(d)] in the present studies. The total peak area for P 2p [Fig. 5(e)] versus irradiation time shows only a small decrease (not more than 10%) during X-ray exposure, indicating DNA strand breaks.

IV. CONCLUSION

In the present studies, we have monitored radiation damage to DNA induced by X rays with XPS. The results show that the DNA molecule is very sensitive to damage by X-ray irradiation and undergoes chemical modifications under exposure. A detailed study of the C 1s, N 1s, O 1s, and P 2p lines in high resolution spectra before and after irradiation has revealed a number of reaction pathways i.e., dehydrogenation, oxygen loss, and formation of radicals, which lead to base damage and/or strand breaks in the DNA molecule. Decrease of peak areas for phosphorus and oxygen in the phosphate and C—OH groups, respectively, indicates a break in the backbone of DNA, while loss of amino and C=O species in studied samples can be attributed to the fragmentation of nucleobases. By finding an increase of signal contributing to particular bonds, the formation of radicals (which are precursors of new products) can be deduced. Additionally, high resolution XPS spectra of the C 1s region for the DNA nucleobases exhibit a range of features that can be uniquely assigned to specific species in the nucleobases.

However, it is known that high energy photons create radiation damage via secondary species including radicals, ions, and secondary electrons.²⁸ Hence, for a deeper understanding of this type of damage, it is necessary to perform a series of experiments on the reactions induced by some of

these secondary species, including the damage induced by the abundant secondary low energy electrons.

ACKNOWLEDGMENTS

S.P. gratefully acknowledges financial support from EPSRC in the form of a Postdoctoral Fellowship at The Open University (EP/D067138/1). The measurements performed in Sherbrooke were supported by the Canadian Institutes of Health Research.

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