■ PAPER ■

# XPS STUDY ON DNA DAMAGE BY LOW-ENERGY ELECTRON IRRADIATION

**Hyung Ah Noh and Hyuck Cho**

Chungnam National University, Physics Department

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After the first report that electrons with sub-ionization energy of DNA could cause single strand breaks or double strand breaks to DNA, there have been various studies to investigate the mechanisms of DNA damage by low-energy electrons. In this paper, we examined the possibility of using X-ray photoelectron spectroscopy (XPS) to analyze the dissociation patterns of the molecular bonds by electron irradiation on DNA thin films and tried to establish the method as a general tool for studying the radiation damage of biomolecules by *low energ y*electrons. For the experiment, pBR322 plasmid DNA solution was formed into the films on tantalum plates by lyophilization and was irradiated by 5-eV electrons. Un-irradiated and irradiated DNA films were compared and analyzed using the XPS technique.

Keywords: DNA damage, XPS, Electron beam, Dissociative electron attachment

## 1. INTRODUCTION

In year 2000, the Sanche group in Canada reported that low-energy electrons (LEEs) with sub-ionization energy of DNA interact with DNA and cause the structural and chemical changes, leading to single strand breaks (SSB) and/or double strand breaks (DSB) through the dissociative electron attachment (DEA) process [1,2]. In their result, SSB was observed as low as at 3-eV incident-electron energy. Since then, there have been many experimental and theoretical studies reported on the mechanisms of DNA damage by the DEA process[2-6]. It is now well established that an LEE with a specific energy resonantly attaches to a constituent of DNA and forms a temporary negative ion, followed by dissociation of the parent molecules, causing a bond break at a specific site of the DNA – this process is called DEA [4-6]. This DNA damage mechanism at sub-ionization energy of DNA is especially important because the secondary electrons are the dominant secondary species generated upon incidence of high energy radiations into the biomolecules. For example, the radiation with incidence energy of 1 MeV deposits its energy to produce about $5 \times 10^4$ secondary electrons, 80% of them having energies less than 20 eV.

In quantifying the SSB and DSB, the gel electrophoresis technique is the one commonly used, as were in the experiments by the Sanche group [1] and by these authors [3]. Even though this is a very useful tool, we cannot tell which bonds of DNA are broken and what kind of fragments are produced from the damages. Therefore, the gel electrophoresis technique has a limit in probing the DNA damage mechanism in detail. To fully understand the damage processes, various different techniques have to be employed.

In this report, X-ray photoelectron spectroscopy (XPS) was used for analyzing the bond cleavage in DNA films with and without LEE irradiation to establish the technique as one of the tools to study DNA damage in various cases.

## 2. MATERIALS AND METHODS

pBR322 DNA extracted from E. coli ER2420 is a commonly used plasmid DNA and was purchased from New England BioLabs Inc. This DNA molecule is a double-stranded and supercoiled DNA with 4,361 bp in length and $2.83 \times 10^6$ daltons in weight. The pBR322 DNA was mixed with TE (Tris-HCl 10 mM, EDTA 1 mM, pH 8.0) buffer to be diluted to a con-

Corresponding author : Hyuck Cho, hcho@cnu.ac.kr
Physics Department, Chungnam National University, Daejeon 305-764, Korea

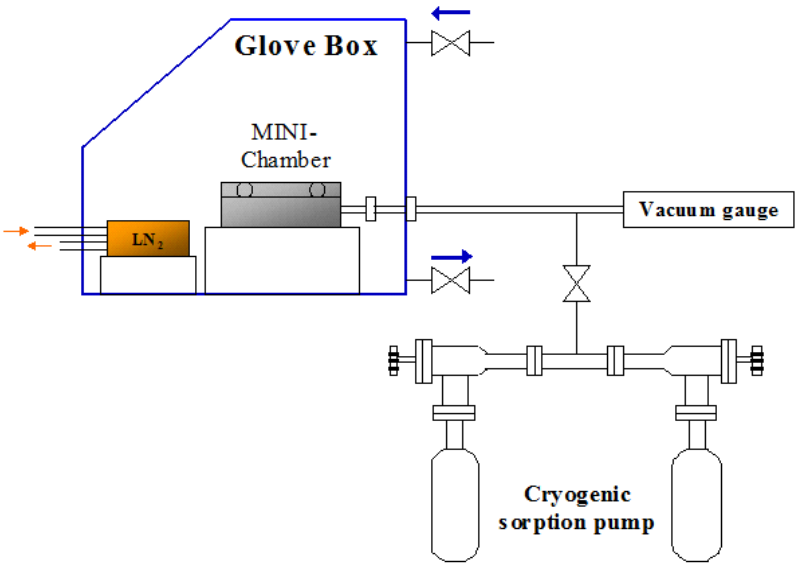


**Figure 1.** Schematic diagram of apparatus for DNA lyophilization.

centration of 1mg·ml$^{-1}$. We used a home-made lyophilizer to prepare dried DNA films on tantalum foils. Lyophilizer as shown in Fig. 1 consists of two main parts a freezer which is using liquid nitrogen for freezing DNA samples and a mini-vacuum chamber equipped with a double-stage oil-free cryogenic sorption pump for drying DNA samples. A drop of DNA solution, with a volume of 30 $\mu$l, was carefully dropped on a chemically clean tantalum foil. The dropped DNA solution was spread on the Ta foil to a circular shape. Immediately after dropping the DNA solution, liquid nitrogen was injected into the freezer and the solution became frozen. The frozen samples were transferred to the mini-vacuum chamber of the lyophilizer system. Water molecules in the DNA solution were extracted by the cryogenic sorption pump. For each run of the experiments, eight DNA films were prepared and loaded into the main vacuum chamber for LEE irradiation. An experimental setup for LEE irradiation on DNA films consists of a homemade electron gun, a Faraday cup, and a sample holder. The sample holder can rotate to irradiate each DNA film separately. When one DNA film was being irradiated, the others were applied with the repelling potential of 9 V to avoid any effects from the stray LEEs. The irradiation of DNA film was carried out under the ultra high vacuum (UHV) condition, ~ $10^{-10}$ torr, pumped by a turbomolecular pump. Each DNA film was irradiated by 5-eV LEE for 5min.

Using the XPS, both the un-irradiated and the irradiated samples were analyzed. XPS is a quantitative spectroscopic technique that measures the composition and the electronic states of the elements of the target material. XPS spectra were obtained by irradiating the material with X-ray beam while simultaneously measuring the kinetic energy and number of electrons that escape. From this information, we could tell the binding energy of the electron ejected from an atom which was bonded to other atoms. We used Thermo Multilab2000 XPS system at the Center for Research Facility, Chungnam National University. This system was equipped with a Mg/Al dual anode beam source, a concentric hemispherical analyzer, and a channeltron detector. The Mg K$\alpha$ source was used for the experiment, and the power and the base pressure were maintained at 150 W and 4x10$^{-10}$torr, respectively. The angle of incidence of the X-ray beam with the sample normal was about 42° and the analyzer was positioned normal to the target surface. XPS spectra were recorded in the fixed analyzer transmission mode with energy steps of 0.1 eV.

When an atom makes a bond with other atoms, its valence electrons are involved in the bonding and their electronic states are changed, which subsequently affect the binding energies of the core electrons. Therefore, a carbon atom, for example, could have different C1s binding energies depending on what kind of bonding it makes with what kind of atoms. This energy shift in the binding energy is called the chemical shift. By exploiting this chemical shift, we could tell that from what kind of chemical bonding the detected electron came from [7].

In this study, we examined the changes in the XPS spectra between the un-irradiated and the 5-eV electron-irradiated samples. And from these changes, we have suggested the possible bond cleavages in the

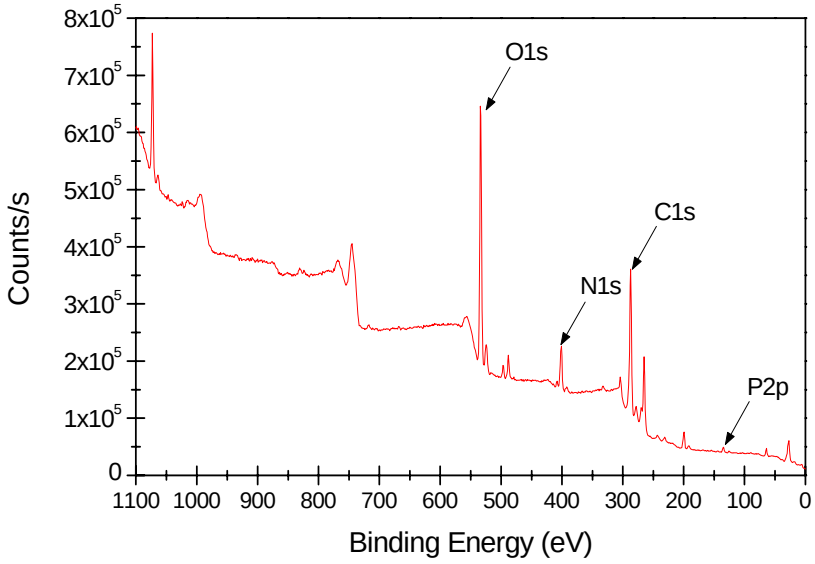


**Figure 2.** XPS survey spectrum of DNA-pBR322 sample.

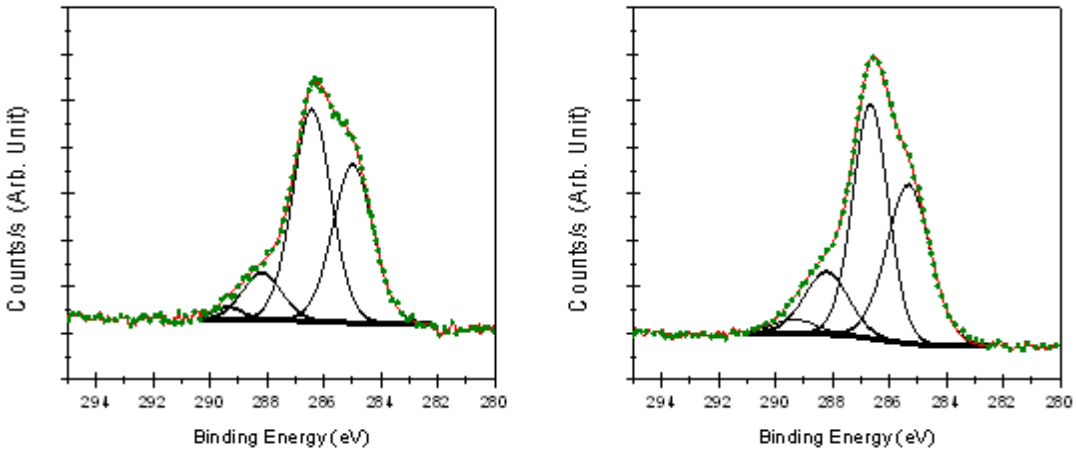


**Figure 3.** XPS spectra of C 1s region for DNA film samples : (a) DNA without electron beam irradiation, (b) DNA with electron beam irradiation. Dots indicate the raw spectra, narrow lines the convoluted peaks, and thick line at the bottom the backgrounds. Counts per second on the horizontal axes are given in the arbitrary units.

DNA samples.

## 3. RESULTS AND DISCUSSION

DNA consists of the bases, sugar and phosphate and has numerous bonds consisting of atoms like H, C, N, O and P. Figure 2 is an example of survey spectrum of an irradiated pBR322 DNA sample, showing the photoelectron peaks from C1s, N1s, O1s and P2p. Photoelectron peak from hydrogen atom is absent in this survey scan, because it cannot be observed by XPS,as well known. Among these peaks, more detailed C1s spectra were scanned for both the un-irradiated and the irradiated DNA targets to confirm the possible damages caused by electron irradiation (see Figure 3). Fig. 3(a) is for the DNA without electron beam irradiation and Fig. 3(b) is for the DNA with 5-eV electron-beam irradiation. However, for a single C1s spectrum, there could be several different contributions from the ejected photoelectrons with slightly different binding energies depending on different bonding of the carbon atom, as mentioned in the previous section. Typical bonding of carbon atom are hydrocarbon bonds [C-H, C-C], carbon bonds to nitrogen [C-N, N-C-N], bonds with oxygen atom [C-O-C, C-OH], amide bond [N-C=O], urea bond [N-C(=O)-N], and others. Binding energies of these bonds are different

from each other and the values are available from the references [8-10] and summarized in Table 1. This means that the spectra in Figure 3 have several components from these different bonds and have tobe deconvoluted accordingly, the results of which are also presented in Figure 3. In the figure, dots indicate the raw C1s spectra, narrow lines the convoluted peaks, and thick lines at the bottom the backgrounds. In Table 1, each constituent peak is assigned to a proper bond. The changes in the sizes (areas) of the constituent peaks between without-irradiation and with-irradiation indicate the bond cleavages of the corresponding bonds. These changes are also tabulated in Table 1. The sizes of some peaks are increased, while othersare decreased by electron irradiation. These changes in size are relative, not absolute – in other words, an increase in peak size does not mean an increase (i.e. new production) in the corresponding bond, because the areas of the spectra are decreased by electron irradiation by about 3～4% in average. However, a decrease in peak size means a decrease (i.e. bond cleavage) in the corresponding bond.

**Table 1.** Binding Energy of C bond and Relative Sizes of Each Bond Peak without and with Electron Irradiation.

| Peak number | Bond type | Center Binding energy (eV) | Relative size of each constituent peak (%) | |
|---|---|---|---|---|
| | | | without irradiation | with irradiation |
| 1 | C-H, C-C | 285 | 37.87 | 37.06 |
| 2 | C-N, N-C-N C-O-C, C-OH | 286~287 | 48.72 | 44.78 |
| 3 | N-C=O | 288 | 11.08 | 14.85 |
| 4 | N-C(=O)-N | 289 | 2.33 | 3.30 |

In the experiment, 16 DNA film samples were made identically – among them, 8 samples were un-irradiated and the rest were irradiated. One standard deviation ($1\sigma$) of the average peak-size changes was about 1%. Therefore, any change less 1% was considered statistically no meaningful. From Table 1 and Figure 3, we inferred the meaning indicated by the reduction in the sizes of the peaks. The 285-eV peak, which is corresponding to C-H and C-C bonds, is decreased from 37.87% to 37.06% in its size. But, since the change is less than 1 % (i.e. one standard deviation) it is hard to conclude that the peak size is surely decreased. On the other hand, the 286-287 eV peak, which is corresponding to C-N, N-C-N, C-O-C, and C-OH bonds, is decreased from 48.72% to 44.78% by 3.94%. This apparent reduction could indicate a cleavage of N-glycosidic bond between deoxyribose and the bases or of phosphodiester bond between deoxyribose and phosphate. But, to conclude this reduction as a cleavage of N-glycosidic bond, we need more supporting evidences such as XPS spectra of N1s. C-O and C-OH bonds also exist at the bond between deoxyribose and phosphate and at deoxyribose ring, which means that the size reduction of the 286-287 eV peak could also indicate a cleavage at the deoxyribose-phosphate bond or at deoxyribose ring.

These interpretations are supported by the experiment of Zheng et al [11], in which oligonucleotide tetramers with the sequence of CGTA or GCAT were irradiated by low-energy electrons and analyzed for the radiation damage using the mass spectrometry technique. They suggested a mechanismof damage involving initial electron attachment to nucleobase moieties, followed by electron transfer to the sugar-phosphate backbone, and subsequent dissociation of the phosphodiester bond. A similar damage mechanism was also suggested by the theoretical study of Li et al [12]. Comparing our observation with the results of Zheng et al and Li et al, it can be concluded that, by scanning the *all the elements* in the target, the XPS technique could be used for a rather complete study of radiation damage in DNA by low-energy electrons.

## 4. CONCLUSION

We examined the possibility of using X-ray photoelectron spectroscopy (XPS) to analyze the dissociation patterns of the molecular bonds by electron irradiation on DNA thin films. pBR322 plasmid DNA extracted from E. coli ER2420 was formed into the films on tantalum plates by lyophilization and was irradiated by 5-eV electrons. Un-irradiated and irradiated DNA films were compared and analyzed using the XPS technique. It was observed that the 286-287 eV peak, which is corresponding to C-N, N-C-N, C-O-C, and C-OH bonds, is decreased statistically significantly. This reduction could indicate a rupture of N-glycosidic bond between deoxyribose and the bases or of phosphodiester bond between deoxyribose and phosphate. To confirm this reduction as a cleavage of N-glycosidic bond, we need more supporting evidences. Since C-O and C-OH bonds also exist at the bond between deoxyribose and phosphate and at the deoxyribose ring, the size reduction of the 286-287 eV peak could also indicate a cleavage at the deoxyribose-phosphate bond or at the deoxyribose ring. By comparing our observation with the results from the previous studies which em-

ployed the techniques different from ours, we conclude that, by scanning the *all the elements* in the target, the XPS technique could be used for a rather complete study of radiation damage in DNA by low-energy electrons. Applying this method more extensively, in future, we are planning to study the combined effects of low-energy electrons and metal ions on bio-molecular damages.

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