EIPAM REPORT

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Reference number

1309

Dates of visit

Starting date: 12.02.2007
Ending date: 23.07.2007 (23 weeks)

The title of your project

Investigation of UV- and low-energy electron induced damage of DNA

1. Purpose of the visit

Ionizing radiations, from natural background radiation or derived from diagnostic and therapeutic techniques, (e.g. X-rays, radiotherapy, positron emission tomography) 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. It is now known that the effectiveness of different ionizing radiations is critically dependent on the *patterns of ionizations* they produce on a nanometre scale, comparable with the diameter of the DNA helix. Theoretical track structure modelling is being used with increasing sophistication to simulate the distinctive patterns of ionizations produced by ionizing radiation. Such models reveal that much of the radiation damage is *site specific* with penetrating primary radiations (i.e. energetic photons or ions) producing nanometre sized clusters of ionizations at the end of the radiation track. Hence in order to understand the mechanism of radiation damage it is essential to understand the interaction of different types of radiation with the constituent cellular molecules - DNA itself and its complements the nucleotides, the nucleosides, phosphates, sugars and, of course, cellular water.

2. Description of the work carried out during the visit

Biological macromolecules, such as proteins or DNA are known to be very sensitive to ionizing radiation. Radiation has a fundamental importance in biology and medicine (e.g. mechanisms of mutagenesis and methods of radiation protection). Radiation-induced chemical modifications in the solid state can be monitored by X-ray photoelectron spectroscopy (XPS), which is sensitive to changes in the overall surface composition and to chemical transformations of functional groups.[1]

Photoemission measurements were performed in a load-locked Kratos XSAM 800 surface analysis system equipped with MgKα and AlKα X-ray sources and a hemispherical energy analyzer. The base pressure of this ion- and turbo-pumped system was 10^{-7}-10^{-8} mbar as read on an uncalibrated, cold cathode gauge. XPS spectra were recorded in the fixed analyzer transmission (FAT) mode with medium pass energy. The magnification of the analyzer in the FAT mode was selected to collect photons from the smallest allowable area on the specimen. The photoelectrons were excited by a dual anode X-ray gun. The X-ray power was kept relatively low (260 W, 13kV x 20mA) so as to minimize sample heating. The detection system of the Kratos XSAM 800 consisted of a single channel multiplier and a fast response head amplifier.[2]

In this experiment a drop of calf thymus DNA solution (10μg/ml) was placed onto a chemically cleaned silicon substrate (0.7 x 0.7 cm), which was very carefully dried. Then the sample was inserted inside a vacuum chamber (10^{-7}-10^{-8} mbar). The DNA was then irradiated by X-rays and the photoelectron current was recorded as a function of time (from 0 to 5h). The electron spectrum obtained is a plot of the number of emitted electrons per energy interval versus their binding energy (eV). Each chemical element has a unique 'electron' spectrum and the spectral peaks from a chemical mixture are approximately the sum of the elemental peaks from the individual molecular constituents. Through the XPS method, we are able to detect Na (1s), O (1s), N (1s), C (1s) and P (2s and 2p) peaks in the constituent DNA. We have observed changes in the photoelectron spectra as a function of irradiation time for some of elements. Such changes are due to chemical modifications of the system under study, e.g. free radical formation or mass loss.

3. Description of the main results obtained

- the XPS spectrum of pristine DNA (dropcast) on silicon substrates is presented in Fig. 1.

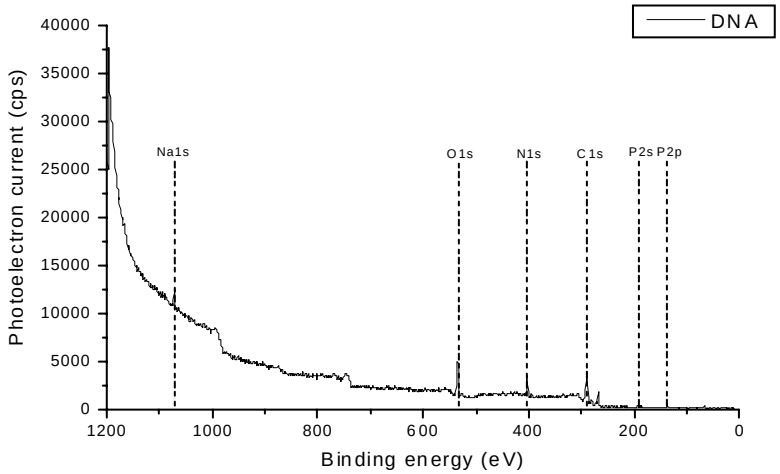

Fig. 1: XPS wide spectrum of a pristine DNA sample

- the XPS spectrum of DNA (dropcast) showed the presence of sodium (Fig. 2), oxygen (Fig. 3), nitrogen (Fig. 4), carbon (Fig. 5) and phosphorus (Fig. 6 and 7).

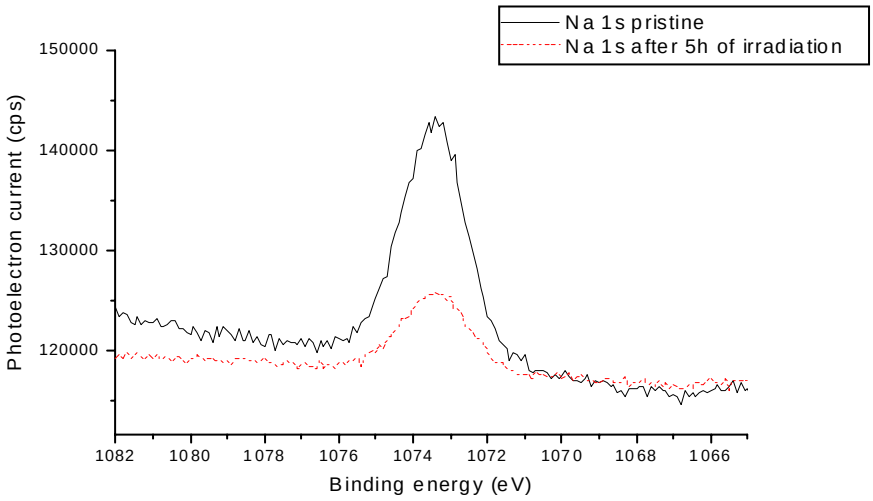

Fig. 2: Detailed Na 1s spectrum of DNA (before and after irradiation)

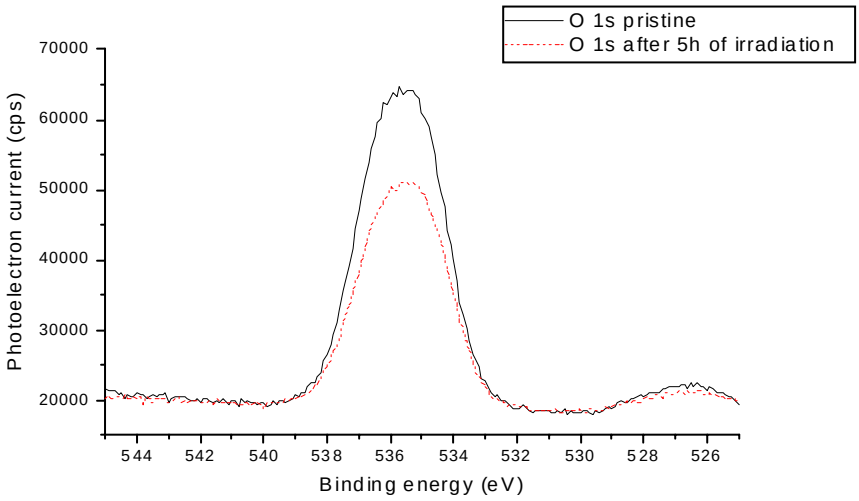

Fig. 3: Detailed O 1s spectrum of DNA (before and after irradiation)

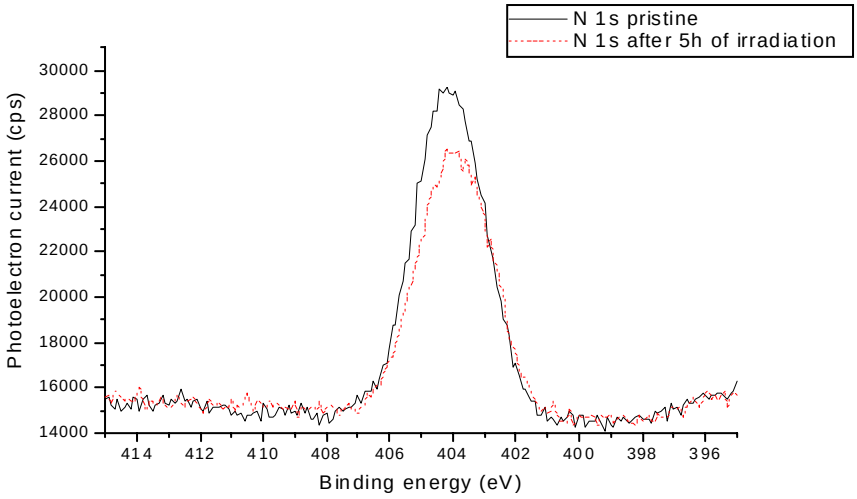

Fig. 4: Detailed N 1s spectrum of DNA (before and after irradiation)

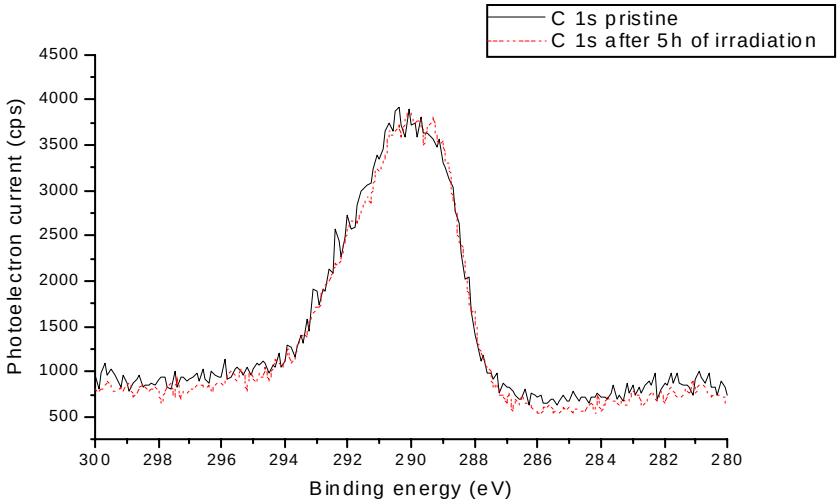

Fig. 5: Detailed C 1s spectrum of DNA (before and after irradiation)

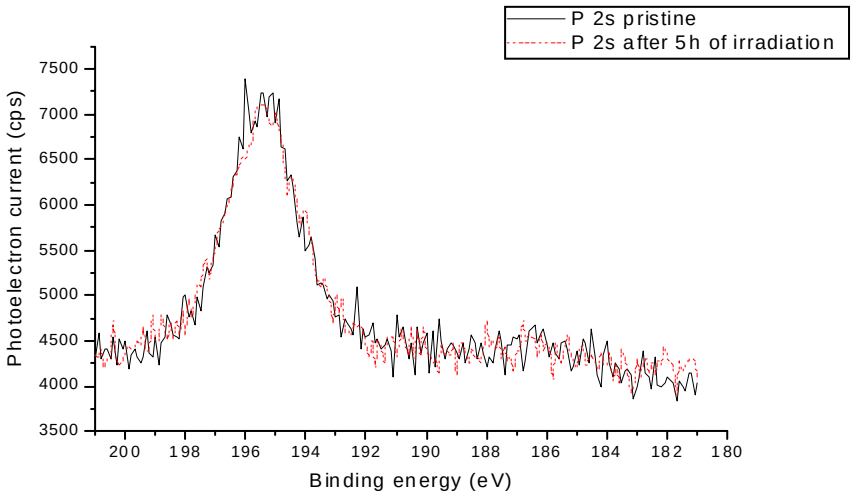

Fig. 6: Detailed P 2s spectrum of DNA (before and after irradiation)

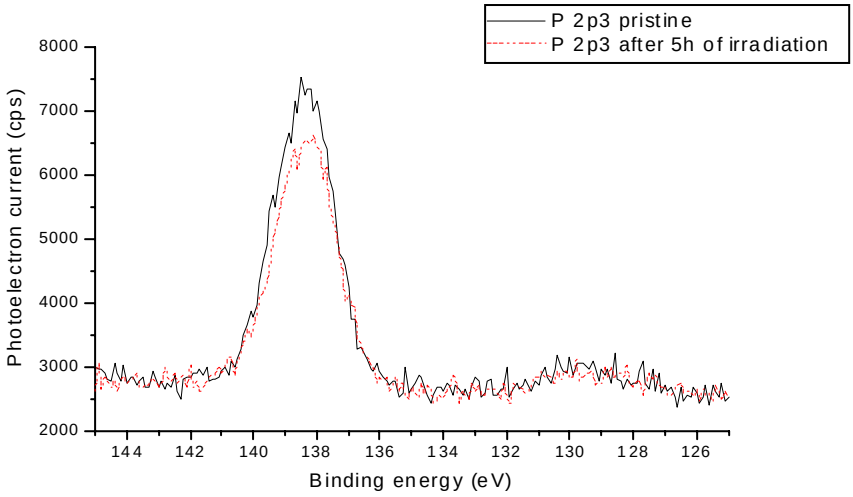

Fig. 7: Detailed P 2p spectrum of DNA (before and after irradiation)

- in all samples the XPS measurements performed before and after radiation exposure showed strong differences in oxygen and nitrogen peaks, with the disappearance of some oxidation states and decrease for some components of carbon and phosphorous peaks.
- it is evident that the peaks height ratio decreased during the irradiation.
- the measured binding energies for C1s closely matched functional group energies corresponding to C=O bonding (288.2 eV) and C-H bonding (283.9 eV), but surface charge masks some bonding.[3]
- it is clear from the XPS data in Figs. 2-7 that the DNA decomposed over a period of 5h during continuous X-ray irradiation.

4. Future collaboration with host institution (if applicable)

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5. Projected publications / articles resulting or to result from your grant

The obtained results, after completion, are to be published in one of the leading journals of the field.

The results were presented at two conferences:

1. P.J. Gomes, A. Stypczynska, P.A. Ribeiro, T. Nixon, N.S. Braithwaite, M. Raposo, Effect of electron beam irradiation in thin films of DNA and DNA nucleic bases, *Radam'07*, Dublin, Ireland, 19th-22nd **2007**

2. A. Stypczynska, S. Ptasinska, T. Nixon, N. Mason, Effect of X-ray radiation on DNA, *XV International Symposium on electron – molecule collisions and swarms*, Reading, United Kingdom, 1st-4th **2007**

6. Other comments (if any)

References

[1] Y. Zubavichus, O. Fuchs, L. Weinhardt, C. Heske, E. Umbach, J.D. Denlinger, M. Grunze, *Radiation Research*, 161, 346-358, **2004**
[2] M.J. Bozack, Y. Zhou, S.D. Worley, *J. Chem. Phys.*, 100 (11), **1994**
[3] D. Briggs, J.T. Grant, *Surface Analysis by Auger and X-ray Photoelectron Spectroscopy*, IM Publications and Surface Spectra, **2003**