O-12 **Session A, Wednesday, 15.06., 12^{20} - 12^{40}**

DNA UV-damage investigated by X-ray spectroscopy

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Keywords: X-ray spectroscopy, XAS, XES, DNA

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X-ray spectroscopic methods are atom-specific techniques, using X-ray excitation to gather information about the electronic and geometric structure of the studied system. In biological research the advantages of X-ray absorption spectroscopy (XAS) are already exploited in wide range of studies, while the use of X-ray emission spectroscopy (XES) in this field is scarce although it is a rapidly developing spectroscopic tool. XAS reflects the unoccupied density of states while XES provides information about occupied states and, when combined, these two methods give detailed picture of electronic structure of studied element.

In the presented studies we demonstrate application of the X-ray spectroscopy to investigate the molecular structure of the damage caused by UV radiation in DNA. Understanding the interaction between radiation and molecules that make up the building blocks of the human organism is crucial for designing effective radiation therapy and protection. DNA, being one of the most important components of the human cell, is an influential target of radiation. Therefore, many fundamental studies have focused on the effects of radiation on DNA.

We used phosphorus K-edge X-ray absorption spectroscopy to study changes in chemical structure around the phosphorus atom of the phosphodiester DNA backbone caused by exposure to radiation. By combining the experimental results with theoretical calculations (see Fig. 1), it has been possible to establish the types and relative ratio of lesions produced by UVA around the phosphorus atoms in DNA. X-ray spectroscopy approach provides information not only about the damage types but also about changes in electronic structure around the phosphorus atoms associated with each damage type, which can help to establish the possible mechanisms involved [1]. The experimental procedure used does not require any sample chemical preparation or treatment, avoiding any additional sample preparation complications that may result in affecting the conclusions.

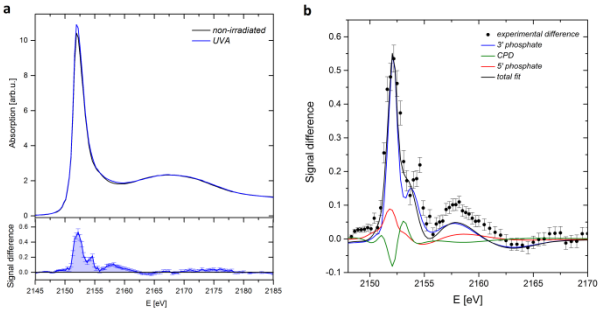

Figure 1. (a) Top: phosphorus K-edge x-ray absorption spectra of intact and UVA-irradiated aqueous DNA samples. Bottom: P K-edge XAS difference signal between the spectrum of damaged and reference DNA samples. (b) Experimental difference of P K-edge XAS obtained for a UVA-irradiated DNA sample fitted with theoretical spectra calculated in FEFF.

XAS studies are extended to XES spectroscopy allowing to acces occupied states of phosphorus site. Non-resonant Kβ X-ray emission spectra of the set of phosphorus compounds were measured with the use of dedicated compact chamber with von Hamos spectrometer setup that enabled us to perform experiment at high energy X-ray beamlines of Swiss Light Source (PSI, Villigen, Switzerland). Kβ lines, in contrast to Kα, provide with high sensitivity information on the chemical environment of the studied atom such as bonding, ligand type and symmetry The results obtained during the experiment showed changes in emission spectra, resulting from changes in chemical structure around phosphorus atom, particularly the number and type of the nearest neighbours of PO_4^{3-} group. Since changes in phosphodiester backbone of DNA caused by radiation are affecting mainly the phosphate groups, our results demonstrate the possibility of using X-ray emission spectroscopy in studies of DNA damage.

Acknowledgments: We acknowledge Swiss Light Source at Paul Scherrer Institute for granting the beamtime. The research leading to these results has received funding from the European Community's Seventh Framework Program (FP7/2007-2013) under grant agreement No. 290605 (COFUND: PSI-FELLOW).

[1] J. Czapla-Masztafiak, J. Szlachetko, C.J. Milne, E. Lipiec, J. Sá, T.J. Penfold, T. Huthwelker, C. Borca, R. Abela, W.M. Kwiatek, *Biophys J.* **110** (2016) 1304.