Application of synchrotron-based FTIR spectroscopy to study DNA damage in single cells irradiated by proton and X-ray microbeams

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The application of microprobe systems to study DNA damage is a very important development for understanding the response of biological systems to radiation exposure [1]. Research of radiation dose-dependent biological effects (such as DNA double strand breaks – the most important DNA damage produced by ionizing radiation) enhances the understanding of the mechanisms leading to cell death [2]. Optimization of SR-FTIR microspectroscopy to study radiation damage in single cells allows fast detection of damage in lipids, proteins and nucleic acids on molecular level at the same time. The aim of this study was the application of SR-FTIR microspectroscopy to investigate the DNA damage in single cells exposed to proton and X-ray microbeams.

The DNA damage in single cells was induced by two types of ionizing radiation: a) protons from the focused horizontal microbeam of the Van de Graaff accelerator and b) X-rays from the 4.5 keV X-rays microprobe. The prostate cancer cells DU-145 were irradiated by specific number (50, 200, 400, 2000, 4000) of protons (with the energy of 0.5, 1 and 2 MeV) and comparable dose of X-rays (0.1, 0.2, 0.5, 2, 5 Gy) per cell. The FTIR analysis of fixed cells was performed at the SR source at SISSI beamline, ELETTRA Laboratory, Trieste, Italy. More than 30 SR-FTIR spectra of single cells for each group were collected. The spectral region of 950 cm^{-1} – 1240 cm^{-1} was subjected to statistical analysis.

Principal Component Analysis (PCA) showed some clustering between all spectra. The hierarchicial cluster analysis (Ward's method) was performed to illustrate the degree of similarity between the averaged spectra of each cell in the same group. The cellular spectra bands (more than 50 ones derived from proteins, nucleic acids and lipids [3-4]) were fitted with Gaussian-Lorentzian curves after the Mie scattering effect correction [5]. The dose-depended changes in the relative intensities of peaks: 960 cm^{-1} (ribose-phosphate skeletal motions), 1084 cm^{-1} and 1095 cm^{-1} (symmetric and antisymmetric stretching of O-P-O band), as well as shape and intensity modification of the 1105 cm^{-1} peak (symmetric stretching of P-O-C band) were observed. The results were compared with spectra simulations generated by HyperChem and Gaussian programs. The Density Functional Theory (DFT) method was appplied for vibration and rotation analysis of damaged DNA molecule such as: DSB - double strand break, SSB – single strand break, DNA-DNA crosslinks, and oxidative damage.

The results obtained with SR-FTIR application allowed to find the correlation between the type, energy and dose of applied radiation and probability of specific DNA damage appearance.

References

[1] W. Polak, O. Veselov, J. Lekki, Z. Stachura, M. Zazula, R. Ugenskiene, M. Polak, Z. Styczeń, *Nucl. Instr. and Meth.* 249, 743–746 (2006).
[2] R. Ugenskiene, J. Lekki, W. Polak, M. Prise, M. Folkard, O. Veselov, Z. Stachura, W.M. Kwiatek, M. Zazula, J. Stachura, *Nucl. Instr. and Meth.* 260, 159 (2007).
[3] G. Socrates "Infrared characteristic group frequencies" Wiley & Sons, New York, (2004).
[4] B. Stuart "Infrared Spectroscopy: Fundamental and Applications" John Wiley & Sons (2004).
[5] P. Bassan, A. Kohler, H. Martens, J. Lee, H.J. Byrne, P. Dumas, E. Gazi, M. Brown, N. Clarke, P. Gardner, *Analyst* 135, 268 (2010).