Journal of Physics: Conference Series **430** (2013) 012040 doi:10.1088/1742-6596/430/1/012040

EXAFS studies of prostate cancer cell lines

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Abstract. Sulphur plays a vital role in every human organism. It is known, that sulphur-bearing compounds, such as for example cysteine and glutathione, play critical roles in development and progression of many diseases. Any alteration in sulphur's biochemistry could become a precursor of serious pathological conditions. One of such condition is prostate cancer, the most frequently diagnosed malignancy in the western world and the second leading cause of cancer related death in men. The purpose of presented studies was to examine what changes occur in the nearest chemical environment of sulphur in prostate cancer cell lines in comparison to healthy cells. The Extended X-ray Absorption Fine Structure (EXAFS) spectroscopy was used, followed by theoretical calculations. The results of preliminary analysis is presented.

1. Introduction

Prostate cancer is recognized as one of the major medical problems facing the male population. Nevertheless, the factors that determine the risk of developing clinical symptoms are not well known [1]. The role of sulphur in prostate cancer progression may be significant for understanding the process of carcinogenesis. In human body sulphur is found mostly as a component of protein-building amino acids (methionine, cysteine and cystine), and thus it participates in a wide range of essential biological functions. J. Mates *et al.* presented function of different compounds containing sulphur in cancer progression. They discussed that cellular methylation and antioxidant metabolism are linked by the transsulfuration pathway, which converts the methionine cycle intermediate, homocysteine, to cysteine, the limiting reagent in glutathione synthesis [2]. Glutathione, on the other hand, has many physiological functions, including its involvement in the defence against reactive oxygen species. Disturbance in this biochemical balance could lead to serious pathological conditions. One of the methods to get information about these transformations is to examine the changes occurring in the nearest chemical environment of sulphur existing in sulphur-bearing compounds in biological systems. In this study the Extended X-ray Absorption Fine Structure (EXAFS) spectroscopy was used in order to establish whether and what changes take place around the sulphur atom in chosen compounds in prostate cancer cells.

EXAFS spectra contain information about the average local atomic environment around sulfur. The spectra can be modeled to determine bonding distances and neighboring atom types and changes that occur to sulfur under different conditions. It has been used since more specific analysis of the local neighbourhood of sulphur atoms can be obtained from such studies. EXAFS is now often used in biology and medicine in the determination of very complex sequences of processes occurring in living organisms. XANES studies of the same material, evaluated before, showed the differences in the ratio of sulphur chemical species between various cell types. Therefore the EXAFS analysis was performed in order to improve the information gained from XANES.

2. Experimental

EXAFS measurements of S K edge were performed at the SUL-X beamline of the synchrotron radiation source ANKA, Karlsruhe (Germany), in fluorescence mode under high vacuum to avoid absorption of the radiation in air. A 7–element Si(Li) fluorescence detector (Gresham, now SGX Sensortech) was used. The experiments on SUL-X beamline can be done at the energy range 2,14 - 20 keV, with a minimum beam size of 30 μm x 25 μm (horizontal x vertical), obtainable by focusing with Kirkpatrick-Baez mirrors and the downsizing horizontal and vertical slit gaps. The dimension of the beam spot used for this experiment was 350 μm x 150 μm and 50 μm x 50 μm for reference compounds and for cell samples, respectively. The beam size of 50 μm x 50 μm was chosen for cell samples in order to obtain the best signal to noise ratio and to be close to the cellular size. The energy was varied with the double crystal monochromator (Si(111) crystal pair) in steps of 5 eV, 2 eV and 0,2 eV in the region of -100 to -50 eV before the edge, -50 to -20 eV before the edge and in the edge region, respectively. The post-edge region was collected to k=9,43 $Å^{-1}$ with k increment of 0,05 $Å^{-1}$. X-rays with higher harmonic energies were suppressed by detuning the monochromator second crystal to about 50% of its maximum intensity. Energy has been calibrated to 2481.4 eV at the maximum of the sulphate white line obtained from the scotch tape.

The experimental material consisted of four commercially available cell lines: three from metastasized prostate cancer (PC3, LNCaP, DU145) and one from the peripheral zone of the prostate, used as a control (PZ-HPV-7). The organic reference compounds were analysed in parallel to provide spectral information of interactions between sulphur and ligands expected, such as oxygen, carbon, nitrogen and other sulphur atoms.

The data were evaluated using the two of the IFEFFIT package programs: ATHENA and ARTEMIS [3]. The spectra were averaged from data collected at six different points on each sample. Firstly, the baseline correction and normalization was performed. Then, the spectra were changed from energy dependence to photoelectron wave number (k). The EXAFS signal $\chi(k)$ was extracted from experimental data, and Fourier transformed from k to R space using the data range from k=2.2 to 7.5 $Å^{-1}$ using a Hanning window. The theoretical amplitudes and phases for the chosen scattering paths were calculated using FEFF8.4 [4].

3. Results and discussion

In case of biological samples, the XANES and EXAFS signal is the average of the contribution from all sulphur atoms present in different cell structures. In this EXAFS studies we focused only on the nearest sulphur neighbours since further coordination shells requires more detailed analysis.

Base on the preliminary analysis of EXAFS data for different cell lines, as it can be noticed on Fig. 1, we can assume that first coordination shells around sulphur will be similar in all studied samples. Main differences are in the shape and magnitude of first and second feature of Fourier transform.

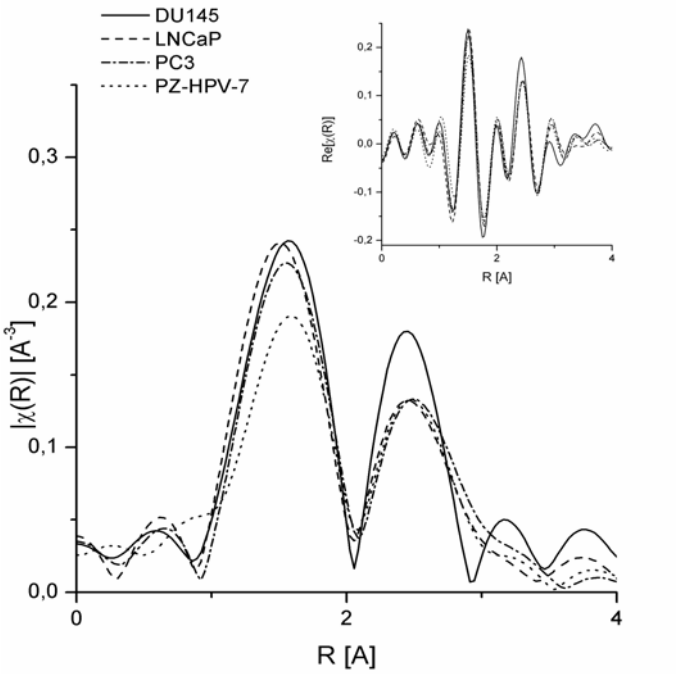

Fig. 1. Fourier transform and its Re part of the sulphur K-edge EXAFS signal for prostate cancer cell lines and control cell line.

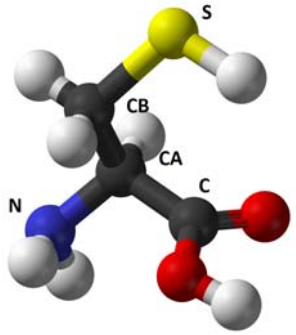

Fig. 2. The structure of cysteine [5].

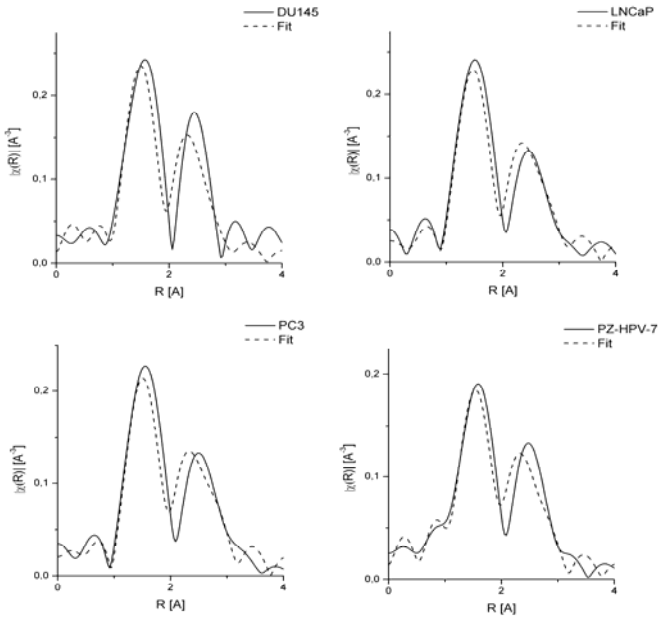

Fig. 3. Comparison of experimental and fitted spectra of different cell lines.

Concerning the results of XANES experiment, performed on the same samples, we determined that the most abundant of sulphur species in cells are amino acids and their content is 60%-75%. For that reason, in order to perform calculations, the crystallographic data for cysteine were used. In cysteine, sulphur is bind directly to the carbon (CB) as can be seen on Fig.2, there are also another two carbon atoms (CA and C) and one nitrogen atom (N) in the nearest neighbourhood. The positions of these atoms were used in theoretical calculations, as the assumption was done that this kind of nearest environment is most likely in studied cells. The coordination number was determined from crystal structure and not included into the fit.

The other tested crystallographic structure was methionine, that is the second common sulphur-bearing amino acid and has two carbon atoms directly bind to sulphur. Nevertheless this calculations didn't give physically meaningful results.

The results of the fits made in ARTEMIS are presented on Fig. 3. The agreement between fitted and experimental data for first two peaks in the case of each sample is fairly good. Additionally the distances between sulphur central atom and its nearest neighbours for different cell lines were determined and the results are presented in Table 1.

Table 1. The distances between sulphur and neighbouring atoms determined during ARTEMIS fits along with parameters of the fit.

Cell line	Calculated parameters			
	S-CB	S-CA	S-N	S-C
DU145	**R [Å] 1,59 ± 0,02** σ^2 [Å^2] 0,029 ± 0,00 ΔE_0 [eV] -7,9 ± 4,8	**R [Å] 2,58 ± 0,02** σ^2 [Å^2] 0,029 ± 0,007 ΔE_0 [eV] -12,8 ± 2,8	**R [Å] 2,98 ± 0,02** σ^2 [Å^2] 0,019 ± 0,018 ΔE_0 [eV] -1,4 ± 1,9	**R [Å] 3,05 ± 0,02** σ^2 [Å^2] 0,029 ± 0,007 ΔE_0 [eV] -7,9 ± 4,8
	S_0^2 0,45 ± 0,31			
LNCaP	**R [Å] 1,61 ± 0,01** σ^2 [Å^2] 0,021 ± 0,007 ΔE_0 [eV] -7,0 ± 2,9	**R [Å] 2,60 ± 0,01** σ^2 [Å^2] 0,018 ± 0,008 ΔE_0 [eV] -13,3 ± 2,0	**R [Å] 2,99 ± 0,01** σ^2 [Å^2] 0,018 ± 0,012 ΔE_0 [eV] -1,2 ± 1,4	**R [Å] 3,06 ± 0,01** σ^2 [Å^2] 0,018 ± 0,008 ΔE_0 [eV] -13,3 ± 2,0
	S_0^2 0,49 ± 0,19			
PC3	**R [Å] 1,62 ± 0,02** σ^2 [Å^2] 0,014 ± 0,005 ΔE_0 [eV] -5,7 ± 3,3	**R [Å] 2,61 ± 0,02** σ^2 [Å^2] 0,010 ± 0,006 ΔE_0 [eV] -14,1 ± 2,9	**R [Å] 3,01 ± 0,02** σ^2 [Å^2] 0,005 ± 0,008 ΔE_0 [eV] -0,6 ± 1,8	**R [Å] 3,07 ± 0,02** σ^2 [Å^2] 0,005 ± 0,008 ΔE_0 [eV] -0,6 ± 1,8
	S_0^2 0,32 ± 0,16			
PZ-HPV-7	**R [Å] 1,62 ± 0,02** σ^2 [Å^2] 0,019 ± 0,006 ΔE_0 [eV] -9,3 ± 3,6	**R [Å] 2,61 ± 0,02** σ^2 [Å^2] 0,017 ± 0,008 ΔE_0 [eV] -13,6 ± 2,7	**R [Å] 3,01 ± 0,02** σ^2 [Å^2] 0,015 ± 0,011 ΔE_0 [eV] -1,4 ± 1,9	**R [Å] 3,07 ± 0,02** σ^2 [Å^2] 0,017 ± 0,008 ΔE_0 [eV] -13,6 ± 2,7
	S_0^2 0,20 ± 0,11			
Theoretical model	1,80	2,80	3,20	3,26

As it is shown, the calculated distances vary from the theoretical values obtained from crystallographic model, but as it was mentioned before this may be related with the EXAFS signal averaging from different sulphur structures. The results for cell lines are similar, the most different values were obtained for DU145 cells and PZ-HPV-7 (control) cells nevertheless this differences are not statistically significant.

4. Conclusions

The use of EXAFS technique combined with theoretical calculations showed that the complex biological systems, such as cells, can be analysed with this method. The model presented, which works fairly well for the measured spectra and contains 3 C atoms and 1 N atom in the nearest neighborhood of sulphur occurring in cells, corresponds to the structure of amino acid cysteine, although the distances between individual atoms are slightly different than in theoretical model of cysteine. For this fitted structure no significant differences in atom distances were found between various cell lines nevertheless, as in the case of XANES analysis, the most diversified results were obtained for DU145 and control cells. That could indicate the fact that the structure of sulphur compounds in DU145 cells is somehow distorted.

Acknowledgements

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement nº 226716. We acknowledge ANKA for granting the beamtime in the proposal BIO-12.

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